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EFFECT OF SEVERE CALCIUM DEFICIENCY ON PREGNANCY AND LACTATION IN THE RAT [1]

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(Received for publication December 28, 1942)

In a previous study (Boelter and Greenberg, '41) it was reported that growing rats reared on a severely calcium deficient ration showed (a) retardation of growth, (b) decreased food consumption, (c) raised basal metabolic rate, (d) reduced activity and sensitivity, (e) extreme demineralization of the skeletal structures, (f) a high death rate, and (g) susceptibility to internal hemorrhages resulting in collapse, prostation and paralysis of the hind limbs.

In the course of this work it was noted that rats which had been maintained from the time of weaning on a diet deficient in calcium failed to mate, although there was no apparent gross or histological change in the reproductive organs. Pregnancy and, more particularly, lactation cause a heavy drain on the calcium and phosphorus stores of the maternal body. This was true in the rat, as has been shown by Goss and Schmidt ('30), even when the diet contained a normal level of calcium and phosphorus. The above considerations led to the study of the effects of severe dietary calcium deprivation on the female animal and its young during gestation and lactation periods.

EXPERIMENTAL METHODS

Twenty-six mature female rats, each of which had borne at least one litter, were transferred from the stock colony ration to the low calcium diet for the purpose of this investigation.

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The diets used were similar in composition to those employed and reported in detail in the previous study of the effect of low dietary calcium on growing rats (Kleiber, Boelter and Greenberg, '40), with the exception of the addition to the food of 1% wheat germ oil. The calcium deficient diet contained 10.6 mg. calcium, 85 mg. magnesium and 425 mg. phosphorus per 100 gm. food; the control diet, which was identical but supplied with adequate calcium, contained 546 mg. calcium, 84 mg. magnesium and 436 mg. phosphorus per 100 gm. food. The same analytical methods were used as in the prior investigations (Boelter and Greenberg, '41).

RESULTS

1. Reproductive success

Production of young from the twenty-six animals, after forty-eight attempts at mating, was noted in only fourteen cases. Only six mothers gave birth to living young. The number of viable young noted in each of these six instances was 1, 2, 3, 7, 7 and 8, respectively. The majority of the remaining mothers ate the young which were dead at birth or which died soon after birth. Six to eight young per litter occurred most commonly. It is possible, where only one to three baby rats were found, that the rest of the litter was eaten by the mother. This conclusion is supported by the fact that the mother's loss in body weight at parturition in each case, no matter what the size of the litter, corresponded approximately to the weight of a litter of eight.

In the majority of cases, two attempts at mating the calcium deficient rats were made. Only one mother was shown definitely to give birth to a litter both times. Her first litter was dead at birth and eaten by her. The second litter consisted of seven living young. Pregnancy doubtless was initiated in other females in the two trials but was not carried through the complete gestational period in both instances. In general, the second attempt at mating was more successful than the first, not only in the number of litters obtained from the group of

rats as a whole, but also in the number of living young obtained per litter. From this fact it is evident that the length of time the rats were maintained on the low Ca diet did not affect materially the reproductive success.

Immature rats, weighing less than 100 gm. at the time they were changed from the stock diet to the calcium deficient diet, after reaching adult age invariably failed to mate on repeated trials. Such rats had an abnormal estrous cycle, indicated by the fact that the vaginal smears showed a continuous diestrous stage rather than the usual cyclical changes. This was evidence of the failure of the deficient animals to gain sexual maturity on reaching adult age. The failure to mate appeared to be the result of actual changes in the reproductive capacity of the rats related to the dietary lack of calcium, but it may have been due simply to the effects of inanition and the general apathy of these animals. Adult female rats transferred to the calcium-inadequate diet after they had reached maturity showed a normal estrous cycle.

Litters of six, eight and ten rats, respectively, were produced by three animals maintained on the control calcium-supplied synthetic diet since the time of weaning. The young were smaller in size than those of the stock colony. The synthetic rations, although they allowed for reproduction and lactation, were not nutritionally optimal. The difference between the stock colony and control young will be pointed out in the ensuing paragraphs.

In 1924 Simmonds observed that rats were able successfully to suckle two litters of young when placed on a diet inadequate in calcium (0.103%) but they subsequently failed to reproduce. Bodansky and Duff ('41), in a study of the effect of various dietary regimes upon the course of parathyroidectomy of the rat, noted that mating of rats was unsuccessful on a low calcium diet (0.017%). Among the different rations employed, the most marked reduction in fertility occurred with the diet lacking in calcium.

2. Symptoms

Even though the rats were deprived of calcium after they were no longer growing, if they were maintained on the inadequate rations long enough they suffered the same consequences shown by young growing rats as the result of calcium deficiency (Boelter and Greenberg, '41). The time of appearance of the symptoms was delayed and the severity lessened for the mature rats. Typical spontaneous paralysis was noted after 12 weeks of calcium deprivation, although severe fatal paralysis did not occur in a group of six female rats for 21 to 35 weeks. Only one death occurred during the gestation period. There were no deaths prior to gestation or during and immediately following lactation.

Convulsive tetany was never observed and could not be induced in either the calcium-deficient mothers or their young.

3. Changes in body weight

Mature female rats failed to maintain their body weight after they were transferred to the low calcium diet. The magnitude of the weight change was slight until the rats had been on the deprivation regime for a long period of time. There was little difference between the weight changes observed among the rats which gave birth to litters and those which did not bear any young. Calcium-supplied control animals maintained their weight after reaching maturity even if subjected to pregnancy and lactation.

The greatest loss in weight occurred at the time of the appearance of spontaneous paralysis in some members of the group. This time also coincided with the period immediately following pregnancy. The demands of pregnancy were probably an additional strain upon the animal causing a loss in body weight and making the rat more susceptible to the consequences of extreme calcium deficiency. A fairly constant body weight was maintained after the initial drop for as long as 40 weeks, unless the rats became paralyzed and prostrate.

Both calcium-deprived rats and their calcium-supplied controls behaved in the usual way with respect to body weight changes as the result of pregnancy. The weight gained during gestation equalled aproximately that lost following birth of the young. The calcium-deficient animals gained an average of 50 gm. during the gestation period and lost 45 gm. at parturition. The controls gained similarly 57 gm. and lost 45 gm. Calcium-deficient mothers which lost weight during the gestation period gave birth to non-living young. The three control females were younger than the deficient ones at the onset of pregnancy, which, furthermore, was the first for the control mothers. The latter animals, in contrast to the deficient rats, had been reared on the synthetic rations from the time of weaning.

Only three calcium-deprived mother rats were maintained on the low calcium diet during the lactation period, and only three young per mother were allowed to survive the whole 28-day interval. During this time each mother lost approximately another 50 gm. body weight. On the other hand, three calcium-supplied control mothers were able to maintain their body weight while nursing three to five young each, and they showed a small gain in weight following the lactation period, as they had not yet reached their maximum weight. It is probable that the calcium-deprived mothers would not have been able to care for the complete litter for the whole lactation period, and certainly the mothers would have lost a greater amount of body weight, if most of the young had not been sacrificed.

The average weight of all the young at birth was normal — about 5.0 gm. At weaning time (28 days) the average weight of the young was as follows: calcium deficient, only 23 gm.; control young, 37 gm.; and stock colony young from a litter of eight rats, 50–60 gm. apiece.

Those calcium-deficient young which were not sacrificed during the lactation period for analytical purposes were maintained on the low calcium diet after weaning. They gained, on the average, 5 gm. the first week and 19 gm. the second

week. After this they lost weight (18 gm.) and most of them died within 2 to 4 weeks after weaning. Control young were maintained on the calcium-supplied synthetic diet for as long as 20 weeks after weaning, during which time they were normal in every respect, except that their growth rate was below that of the stock colony young. The calcium-deficient young showed no symptoms of abnormality, except their minute size, until they suffered induced paralysis. The galvanic stimulus produced instant collapse and paralysis within 10 days of weaning, followed within a few days by the death of the animal. Autopsy showed a typical calcium deficient hemorrhagic condition (Boelter and Greenberg, '41).

The small size of the calcium-deficient young at weaning probably is due to their partial starvation as the result of the poor milk supply of the low calcium mothers. Apparently the calcium deficiency of the mother does not affect the development of the young in utero, but it does hinder materially the growth of the infant rats after birth. The death of many of the young rats soon after birth is to be attributed to the failure and inability of the calcium-deprived mother to care for them.

4. Food consumption

The first few weeks after being transferred to the experimental rations, the mothers consumed only 4–6 gm. of the calcium-low food per rat per day, which is the usual amount for a rat on such a dietary regime. However, the food consumption rose subsequently to normal (9–11 gm. per rat per day) and during pregnancy and lactation was at an even higher level (13–15 gm. per rat per day). The increased amount of food eaten corresponds to that consumed by the control mothers under similar circumstances. At least 10 gm. of the calcium-supplied synthetic diet per rat per day was eaten by the control rats under ordinary conditions. Calcium-deficient weaned young consumed only 2–4 gm. of the deficient ration per rat per day.

5. Chemical composition

Hemoglobin. After giving birth to young the hemoglobin content of the blood of both calcium-deficient and control mothers was below normal. Calcium deprivation had a more pronounced effect on the hemoglobin, however. The amount of hemoglobin of both the deficient and control mothers decreased during the lactation period and was restored to normal about 4 weeks after weaning. Calcium-deficient and control young presented the same blood hemoglobin picture. As the rats grew older, the hemoglobin gradually rose from a low value at birth to the customary level reached at 6–7 weeks of age. Table 1 shows the changes in hemoglobin content of the blood as the result of pregnancy and lactation.

TABLE 1

Hemoglobin concentration of the blood [1]

	MOTHERS		YOUNG	
	Calcium low	Control	Calcium low	Control
Before pregnancy	12.7 (13) [2]	13.5 (3)		
At birth	9.8 (12)	11.3 (7)	9.3 (4)	8.5 (6)
At weaning	8.8 (2)	10 6 (2)	11.3 (6)	11.3 (5)
Four weeks after weaning	12.7 (2)	13.2 (2)	13.0 (2)	13.5 (2)

[1] The data obtained by the Newcomer method ('23) represent average values expressed as grams hemoglobin per 100 ml. blood. The length of time the mothers were maintained upon the calcium deficient diet varied from 26 to 247 days.

[2] The number of cases from which the mean is calculated.

Serum calcium. The serum calcium concentrations are recorded in table 2. The concentration of serum calcium of mature female rats was maintained at the normal level, even though they were deprived of calcium for as long as 275 days. However, if rats were subjected to the additional strain on their calcium stores as the result of pregnancy, the serum calcium concentration was reduced, but never to the low value observed for young growing rats. During the lactation period there was a further slight decrease in the serum calcium concentration of the deficient mothers. The level of the serum calcium of the control mothers never fell below the normal

range. The average serum phosphorus concentration of calcium-deficient mothers was 3.9 mg. per 100 ml. Bodansky and Duff ('41) report similar values for mothers maintained on a low calcium diet (0.017% Ca). The level of the serum calcium concentration of the deficient mother's young remained essentially normal, although it tended to decrease during lactation and was below the corresponding value for

TABLE 2

Serum calcium concentration.[1]

	CALCIUM LOW		CONTROL	
	Range	Mean	Range	Mean
Mothers				
Before pregnancy[2]	9.2–11.0 (5)[3]	10.0±0.30[4]		
Immediately post partum[5]	7.1– 9.4 (5)	8.2±0.40	9.1–10.7 (6)	10.3±0.24
During lactation[6]	6.9– 8.9 (5)	7.9±0.33		
Recovered after pregnancy[7]	9.2– 9.5 (2)	9.4±0.16		
Young[8]	8.9–10.2 (7)	9.6±0.17	10.0–12.3 (8)	10.9±0.29

[1] The data are expressed as mg. Ca per 100 ml. serum. The determinations were made upon individual rats by the method of Greenberg and Miller ('41). Approximately 0.05 ml. serum, obtained from the tail without sacrificing the animal, was used for each analysis. We wish to acknowledge to W. D. Miller our appreciation for carrying out the individual serum calcium analyses.

[2] Mothers, after giving birth to one litter, were transferred from the stock colony to the low calcium diet for a period varying from 153–275 days, during which time they were mated twice without success.

[3] The number in parentheses indicates the number of cases from which the mean was calculated.

[4] Mean deviation of the mean.

[5] Mothers had been on the low calcium diet for a period varying from 80–168 days. In only two instances were the deficient young alive at birth. The control mothers gave birth to living young.

[6] Data from two mothers that nursed three young each. Mothers gave birth to eight and seven rats after 39 and 117 days on the calcium-low diet, respectively. Analyses performed on blood drawn between 2–29 days after birth of young.

[7] Analysis performed 43 and 221 days after mothers gave birth to a litter of seven living and seven non-living young, respectively. The first mother nursed three young for a 4-week lactation period.

[8] Age of the young rats varied from 2–39 days for the deficient, and 2–46 days for the control animals.

the control young. Presumably the mother was able to supply enough calcium in her milk for the serum calcium to be maintained at an almost normal level in the young.

Body calcium. As a result of dietary calcium deprivation, the amount of calcium in the carcasses of mature female rats was reduced to the same extent whether or not they bore young. The decrease in total body calcium was gradual. Pregnancy did not appear to influence markedly the reduction in the calcium percentage, but low values were found following lactation. The length of time the rat was maintained on the calcium-inadequate diet was a more important factor than pregnancy and lactation in the determination of the amount of body calcium. The phosphorus content of the entire carcass of the calcium-deprived rat remained at the normal level. Control mothers showed normal body calcium and phosphorus contents after pregnancy and lactation (table 3).

The body calcium content was lower than normal in the young born of mothers fed the low calcium diet. The percentage of calcium gradually increased until at weaning time (28 days of age) it was almost at the normal level. The amount of body calcium of control young rats continued to increase with the growth of the animal, but that of the deprived young even fell off after 4 weeks of age, so that there was a decided difference in the calcium content of 6- and 7-week old deficient and control rats. The former had only about two-thirds the normal body calcium percentage. These differences are illustrated in table 3.

The total phosphorus content of infant calcium-deficient and control rats increased gradually with the growth of the rats. The low calcium young had a phosphorus percentage slightly lower than that of the controls, but it was nevertheless within the normal limits reported by other workers. There was no significant difference between the total body calcium and phosphorus contents of stock colony young and the infant rats reared on the control synthetic diet.

The fetuses from two calcium-deficient mothers just before term had very low calcium but almost normal phosphorus per-

TABLE 3

Total body calcium and phosphorus [1]

	CALCIUM-DEFICIENT		CONTROL		STOCK COLONY	
	Ca	*P*	*Ca*	*P*	*Ca*	*P*
Mothers		550[2](8)				
After 14 weeks on low calcium diet [3]	816 (6)[4]					
After 28 or more weeks on low calcium diet [5]	596 (10)					
No young while on low calcium diet [6]	691 (8)					
After birth of young [7]	727 (6)					
After lactation [8]	573 (2)		1014 (3)	639 (3)		
Young						
Fetuses	80 (2)	268 (2)				
At birth, living	206 (5)				247 (2)	285 (2)
At birth non-living	129 (5)					
Age in days						
2	199 (2)		336 (4)		273 (2)	325 (2)
7	373 (2)				412 (2)	355 (2)
14	381 (2)	518 (2)	545 (2)		476 (2)	423 (2)
28	773 (3)	535 (3)	703 (2)		820 (2)	627 (2)
42	667 (2)	593 (2)	1065 (2)	675 (2)	977 (2)	627 (2)
56	738 (2)	567 (2)	1121 (2)	647 (2)		

[1] Values are averages expressed in milligrams per 100 gm. fresh rat carcass.

[2] As there was no variation in the total body phosphorus among the categories listed, this figure is the average value for all the calcium-deficient mothers.

[3] This time interval includes a period of pregnancy for each mother and a lactation period for one mother.

[4] The number in parentheses indicates the number of cases from which the mean was calculated.

[5] This group includes a period of pregnancy for two mothers and a period of lactation for one mother. The other rats were mated without success.

[6] These animals were maintained on the low calcium diet for a period varying from 75–247 days, during which time they were mated without success.

[7] These mothers were maintained on the low calcium diet for a period varying from 80–153 days. All gave birth to litters but did not nurse them.

[8] These two rats gave birth to young after 29 and 117 days of calcium deprivation and were sacrificed after 96–287 days of calcium deprivation, respectively.

centages; they would probably not have survived. Apparently only those fetuses which had an almost normal calcium content were carried successfully to term.

This statement is supported by the fact that the calcium-deficient infants that were dead at birth showed a much lower body calcium content than those which survived (table 3). The viable young had only 17% less calcium than the stock infants at birth, whereas the non-living young contained about one-half the normal amount of calcium.

Bone composition. The bones of the control mothers had a normal mineral composition. Furthermore, female rats which were transferred to the calcium deficient diet after having reached maturity had essentially the same percentages of ash, calcium, magnesium and phosphorus in their bones as did the control animals. However, rats which had been maintained on the low calcium diet for a long period of time showed a slight reduction in the per cent of ash and of the calcium in the bone and ash; calcium-deprived mothers showed definite decreases in these respects only if they lactated their young. Pregnancy alone only produced a small decrease in the ash content of the bones. The analytical values obtained for the adult rat bones are recorded in table 4.

According to Bodansky and Duff ('41) demineralization of the bone occurs following pregnancy and lactation, especially if the diet is inadequate in minerals. That even a normally fed rat may draw upon the calcium and phosphorus reserves of the trabeculae of the long bones in response to lactation is seen from the observations of Goss and Schmidt ('30) that almost invariably a negative calcium balance occurs during lactation.

The analytical data on the composition of the bones of the young from calcium-deficient and control mothers are recorded in table 5. The composition of the bone ash of the control young showed the usual calcium, magnesium and phosphorus relationships. However, the per cent of bone ash, and, therefore, the percentages of calcium, magnesium and phosphorus in the bones of the young were low at birth and only tended

TABLE 4
Composition of bone of adult rats.[1]

ANIMALS		ASH	CALCIUM		MAGNESIUM		PHOSPHORUS	
		% of bone	% of ash	% of bone	% of ash	% of bone	% of ash	% of bone
Calcium-deficient mature females — no young (7)[2] (75–247 days)[3]	Range	62.2 – 69.0	32.4 – 39.3	21.3 – 25.8	0.49 – 0.82	0.34 – 0.51	13.7 – 18.0	8.7 – 11.7
	Mean	65.8± 1.1	36.5±0.87	24.0±0.66	0.69±0.048	0.46±0.03	15.9±0.59	10.4±0.42
Calcium-deficient mothers — after birth of young (4) (85–153 days)	Range	58.0 – 66.5	35.8 – 39.6	22.7 – 29.1	0.68 – 0.98	0.43 – 0.65	15.3 – 16.4	8.0 – 10.4
	Mean	62.3± 2.0	38.0±0.80	25.0±1.5	0.79±0.066	0.49±0.053	15.8±0.23	9.8±0.35
Calcium-deficient mothers — after lactation (3) (96–146 days)	Range	48.2 – 56.2	31.8 – 36.7	15.9 – 20.6	0.55 – 0.97	0.31 – 0.49	15.8 – 18.4	7.6 – 10.3
	Mean	51.6± 2.4	33.8±1.5	17.3±1.6	0.75±0.12	0.39±0.054	17.5±0.85	9.0±0.78
Control mothers — after lactation (3) (111–165 days)	Range	61.7 – 72.6	35.5 – 38.7	21.9 – 28.1	0.68 – 0.75	0.44 – 0.54	16.3 – 17.0	10.6 · 12.3
	Mean	66.0± 3.3	37.2±0.93	24.6±1.8	0.73±0.024	0.49±0.029	16.7±0.22	11.9±0.52

[1] The bone analyses were made upon the two femurs of the rat. Data are expressed as per cent of dried, defatted bone and of ash.
[2] The number in parentheses indicates the number of cases from which mean was calculated. Measure of variability is mean deviation of the mean.
[3] The time on the experimental diet expressed in days.

TABLE 5

Composition of bone of young rats[1]

AGE IN DAYS	ASH	CALCIUM		MAGNESIUM		PHOSPHORUS	
	% of bone	% of ash	% of bone	% of ash	% of bone	% of ash	% of bone
			Calcium-deficient young				
0	17.5	49.8	8.72	1.29	0.23	18.1	3.2
10	20.2	35.2	7.10	0.89	0.18	12.5	2.5
16	35.4	24.6	8.69	1.20	0.43	16.0	5.6
22	25.0[2]	33.6[2]	8.40	0.95[2]	0.24	20.3[2]	5.1
29	25.0[2]	33.8[2]	8.45	1.13[2]	0.28	17.3[2]	4.3
29	34.0	40.2	13.80	0.79	0.27	15.0	5.2
39	24.0	34.3	8.23	0.83	0.20	21.5	5.2
45	25.0	32.2	8.04	0.94	0.24	20.5	5.1
57	18.9	45.6	8.62	1.17	0.22	17.6	3.3
			Control young				
11	57.2	33.0	18.9	0.60	0.34	15.8	9.0
11	56.9	39.6	22.5	0.71	0.40	15.5	8.8
28	41.5	38.1	15.8	0.79	0.33	18.8	7.3
32	46.2	39.6	18.3	0.72	0.33	17.2	8.0
46	55.3	43.2	23.9	0.74	0.41	18.3	10.1
47	61.7	37.0	22.8	...	...	17.3	10.6
53	64.5	41.2	26 6	0.67	0.43	15.6	10.1

[1] The bone analyses were made upon the two femurs of the rat. Data are expressed as per cent of dried, defatted bone and of ash.

[2] Very low ash percentages were obtained (10.8 and 9.3%, respectively) for these analyses. As these appeared to be in obvious error, due to loss of ash during the preparation of the bones for analysis, the average value for the per cent of ash of the remaining results was substituted for these two figures in the calculation of the amount of calcium, magnesium and phosphorus in the ash.

to approach the adult level with the growth of the rats. The results are comparable to those obtained by Orent, Kruse and McCollum ('34) for the composition of normal bone as influenced by the growth of the rat.

The bone analyses of the low calcium young presented an interesting picture. The percentage of ash was extremely low at birth. It subsequently showed a slight rise while the rats were being fed by the mother, and then dropped off to the previous level after the rats were weaned. The bones of these young rats were practically indistinguishable from the soft tissues of the body. They were almost transparent and simply

shells for the bone marrow. They were not even comparable in consistency to normal cartilage, but were much less firm and thinner. Normal rat bones at birth, of course, are mainly cartilaginous, and most of the calcification as well as the ossification occurs during the growth of the rat. Because of the minute size of the young bones and their extreme fragility of texture, it was not easy to prevent loss of ash during the preparation of the bones.

The percentages of calcium and phosphorus in the bone ash were about normal, and the percentage of magnesium somewhat higher than normal. This agrees with the observation of Hammett ('23) that the calcium, magnesium and phosphorus make-up of bone ash is always approximately the same regardless of the amount of ash present in the bone. There is a strong tendency for the organism to maintain a balance between the various mineral elements, so that the ash is almost uniform in percentage composition. Bodansky and Duff ('41) also have noted that the bone ash composition is constant under variable conditions of diet. These findings are favorable to the hypothesis that the mineral matter of bone is made up, in the main, of a single, complex compound.

On the other hand, the calcium percentage of the bone at birth was only about one-third the normal adult value. This same percentage of calcium was maintained by the animals throughout their growth. No more calcium was added to the bones of the calcium-deprived young during the lactation period or during the first few weeks after weaning. After the young rats were deprived of their source of calcium from the mothers' milk, they had to use their own meagre stores of calcium in order to meet life's needs. Hence, the percentage of bone ash was markedly reduced after weaning. The amounts of magnesium and phosphorus in the bones were also much lower than the corresponding control values. The whole general bone picture of the low calcium young was similar to that observed for growing rats transferred to the calcium-inadequate diet after weaning. The differences between the control and deficient bones were greatly accentuated, however,

in the case of the young rats from mothers also deprived of calcium.

Cox and Imboden ('36) note that female rats, even though maintained on experimental diets of widely variable mineral content, nevertheless give birth to young having constant mineral composition. Their conclusion is that a change in dietary minerals is not immediately reflected by change in the composition of the fetal tissue, and, conversely, that the maternal organism is able to regulate the mineral elements supplied to the fetus. Furthermore, gestation per se is little strain on the rat mother as compared to the human mother, because rat young are cartilaginous at birth. However, a 21-day old rat approximates the human infant at birth with respect to its calcium skeletal content, so that lactation is definitely an added strain upon the rat mother. On the other hand, Bodansky and Duff ('41) point out that the average litter weight at birth is one-fifth to one-fourth the weight of the mother rat, so that the total calcium of a normal litter is about 5% of the maternal total calcium, whereas the total calcium of a human infant is only approximately 1.5–2.5% of the maternal calcium.

The observations made in this study indicate that lactation is decidely a greater strain than is pregnancy upon the calcium stores of the female rat. However, it should be pointed out that in only a small per cent of the cases of the present investigation was the deficient mother able to provide the normal requirement of calcium for the fetus and, hence, give birth to normal living young. Furthermore, the drain on the calcium stores of the mother during lactation was reduced, not only by the lack of deposition of calcium in the bones of the baby rats, but also by the restriction of the size of the young rats. The maternal organism attempted to produce normal young insofar as that was possible and still maintain its own essential calcium supply.

SUMMARY

1. Rats reared from weaning time on a synthetic diet containing only about 10 mg. calcium per 100 gm. food failed to mate.

2. After being transferred to the diet low in calcium, fertility was markedly decreased among a group of mothers which had borne a previous litter each. The number of viable young produced was very low.

3. The mothers and young were subject to the consequences of extreme calcium deficiency as evidenced by their susceptibility to hemorrhages, prostration and paralysis induced by a galvanic stimulus. The symptoms were greatly exaggerated in severity for the young.

4. Pregnancy was not a great drain upon the calcium stores of the mother rat, but lactation definitely reduced the amount of skeletal calcium. This was shown by a lowering of total body calcium content, serum calcium concentration, percentage bone ash and per cent of calcium of bone and ash. These changes were magnified by a marked loss in weight by the mother during lactation as contrasted to a maintenance of body weight during gestation.

5. Calcium-deficient young that were able to survive, were almost normal at birth except for a low bone ash and bone calcium content. The deficient mother was able to supply some calcium to the young during the lactation period but not enough to maintain the normal calcium content of the skeletal structures. She was not capable of providing enough milk to allow for the normal growth of her young.

LITERATURE CITED

BODANSKY, M., AND V. B. DUFF 1941 Effects of parathyroid deficiency and calcium and phosphorus of the diet in pregnant rats. J. Nutrition, vol. 21, p. 179.

BOELTER, M. D. D., AND D. M. GREENBERG 1941 Severe calcium deficiency in growing rats. I. Symptoms and pathology. II. Changes in chemical composition. J. Nutrition, vol. 21, pp. 61, 75.

COX, W. M., AND M. IMBODEN 1936 The role of calcium and phosphorus in determining reproductive success. J. Nutrition, vol. 11, p. 147.

GOSS, H., AND C. L. A. SCHMIDT 1930 Calcium and phosphorus metabolism in rats during pregnancy and lactation and the influence of the reaction of the diet thereon. J. Biol. Chem., vol. 86, p. 417.

GREENBERG, D. M., AND W. D. MILLER 1941 Severe calcium deficiency in growing rats. III. Serum calcium of individual animals during development of calcium deficiency. J. Nutrition, vol. 22, p. 1.

HAMMETT, F. S. 1923 Studies of the thyroid apparatus. XVII. The effect of thyroparathyroidectomy and parathyroidectomy at 100 days of age on the calcium, magnesium and phosphorus content of the ash of the humerus and femur of male and female albino rats. J. Biol. Chem., vol. 57, p. 285.

KLEIBER, M., M. D. D. BOELTER AND D. M. GREENBERG 1940 Fasting catabolism and food utilization of calcium-deficient rats. J. Nutrition, vol. 19, p. 517.

NEWCOMER, H. S. 1923 A new optical instrument for the determination of hemoglobin. J. Biol. Chem., vol. 55, p. 569.

ORENT, E. R., H. D. KRUSE AND E. V. MCCOLLUM 1934 Studies on magnesium deficiency in animals. VI. Chemical changes in the bone with associated blood changes, resulting from magnesium deprivation. J. Biol. Chem., vol. 106, p. 573.

SIMMONDS, N. 1924 Observations on reproduction and rearing of young by the rat as influenced by diet. Amer. J. Hygiene, vol. 4, p. 1.

DIGESTION OF WHOLE WHEAT AND WHITE BREADS IN THE HUMAN STOMACH *

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The experiments of Russell and Nasset ('41) and of Bly, Heggeness and Nasset ('43) from this laboratory have demonstrated that both motility and secretory activity of the upper gastrointestinal tract in dogs are plainly affected by certain of the B-complex factors, especially pantothenic acid. Animals deficient in this factor required a longer time to evacuate a meal and to digest it to the same degree than did animals receiving whole yeast. It occurred to the writers that one reason why many people do not learn to appreciate whole wheat bread might be a relative paucity or unavailability of pantothenic acid in such breads as compared with white breads. If this factor were essential to gastric motility or to the normal rate of secretion, if indeed it should prove to be an important secretogogue, for man also, slow digestion resulting from its lack might contribute to the palling of appetite for a food with this deficiency.

One way of testing such an hypothesis would be to compare in human subjects the rates of gastric digestion of a whole wheat bread eaten both with and without the addition of Ca-pantothenate. The latter rates might also be compared with those for the same foodstuffs in the same subjects eating equivalent amounts of white breads.

It would obviously be important in testing the rate of digestion of starch that the same length of time be consumed in chewing the several breads, and, for comparison of any speci-

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fied digestion, that reasonably equal amounts of the gastric contents be recovered at the same interval after eating.

Because the laboratory had already gained considerable knowledge of the "peeled-wheat" bread (Murlin, Marshall and Kochakian, '41; Sealock, Basinski and Murlin, '41), it was chosen to represent the truly whole wheat breads in this study, for the flour from which it is baked has had nothing removed except the outermost epidermis of the whole grain, and no other source of starch or protein is added. For comparison, the same bread baked with high-vitamin [1] yeast, and two white breads — one made of 76% extraction flour plus 3½% dry milk solids and baked with high-vitamin yeast, and one made of lower [2] extraction flour containing a somewhat lower percentage of dry milk solids and baked with ordinary yeast — were chosen.

EXPERIMENTAL

Six reliable graduate students were selected as subjects. It was planned to give test meals of peeled-wheat bread to each of these subjects over a period of days until reasonably constant results were obtained as regards the soluble N and free sugar found in samples taken at 1-hour intervals from the time of eating. At the start, the Rehfuss tube was swallowed before the test meal was eaten so that freedom from solids remaining in the stomach could be confirmed. The tube was then left in place while the test meal of bread was taken and for 1 hour longer. However, it was necessary to abandon this procedure for four reasons aside from the difficulty of accomplishing a thorough mastication with the tube in place: (1) Three of the subjects began to develop low gastric acidity within the first week and two of them reached the point of having no free acid in the second week; (2) the "bucket" of the tube became completely clogged so that a freely flowing sample could not be drawn without using excessive quantities of water, which was obviously undersirable; (3) the x-ray pictures taken after barium-mixed meals on days alternating

[1] See p. 133 for latest analysis of this yeast.

[2] About 72% of the entire wheat berry, according to the manufacturer.

with the digestion-test days showed some irregularties of motility attributable, it was thought, to variation in lodging place of the "bucket"; (4) two of the subjects experienced considerable pain and traces of blood were found in their gastric contents. These two persons therefore were excused. Two fresh subjects were selected and a new start was made, after the lapse of a week, to afford a complete rest for the four veteran subjects who had experienced no symptoms.

Under the new regime each subject was instructed to drink a glass of water immediately on arising on the test day and to report at the laboratory in time to begin eating the test bread approximately 1 hour after taking the water. This schedule was observed regularly throughout the remainder of the study. To avoid possible injury to the gastric mucosa from too frequent use of the stomach tube, the tests from June 15 on were limited to 4 successive days the first week and 3 each week thereafter. The subjects started to eat the new bread for each week on Monday at the rate of two slices each meal. The test meal consisting of 150 gm. (five to six slices) fresh weight of each bread, together with 250 ml. of unsweetened clear tea, was given Wednesday [3], Thursday and Friday mornings. At the other two meals on these days the regular two slices were eaten. On Saturdays and Sundays the special breads were omitted and laboratory work was not done. This schedule was tolerated perfectly.

The actual eating of the test meal each time occupied just 30 minutes, with one single exception. This provided none too much time for thorough insalivation but was adhered to for the sake of regularity.

In the second period each subject was given two measured doses of 16 mg. each of Ca-pantothenate with instruction to take one of them on retiring the night before the test meal was to be taken and another 1 hour before the time fixed for the test meal, early in the morning (see footnote 6).

As nearly as possible, 1 hour after completion of the meal the sample of grastric contents was drawn through the Rehfuss

[3] Except for the first period, June 16 to 19, when the test meal began on Tuesday.

tube which had been swallowed immediately before. Variations from the 60 minutes' standard interval were occasioned by plugging of the tube. Corrections were made for these variations as well as for the preformed soluble N and free reducing substances in the breads for the purpose of statistical analysis of the results (see below).

METHODS

After the removal of aliquots for pepsin determination by the Mett method, all samples were immediately boiled to destroy enzyme action, then homogenized, diluted to precise volumes and partitioned for determination of total solids, total acidity, free acidity, total reducing substance after hydrolysis with HCl, free reducing substance, total nitrogen, and soluble nitrogen, by well-known standard procedures.

RESULTS

In table 1 are shown the results of the gastric analyses averaged for the 3 (or 4) days in each period for each subject. The values for any given substance shown by any single individual were surprisingly consistent from period to period. For example, the amount of total solids in the sample was always highest in the gastric contents from subject 6. This was due to a large percentage of mucus. The highest total acidity was shown in every period by subject 2, with subject 1 always a close second. These two subjects also showed the highest free acidity, while the lowest value on peeled-wheat breads appeared in the sample for subject 6. Subject 2 exhibits in every period the lowest average free sugar, a circumstance doubtless related to his high free acidity. It is reasonable to suppose that the low pH in the gastric contents retarded starch digestion by ptyalin. Correspondingly the highest values for free sugar in the gastric contents accompany low values for free acidity. High values for pepsin would be expected to accompany high free acidity and such proved to be the case. Further, low values were associated with low acidity and medium values with medium acidity.

TABLE 1

Analysis of stomach contents.

SUBJ.	VOL. OF SAMPLE	TOTAL SOLIDS	TOTAL ACIDITY	FREE ACID	REDUCING SUBSTANCE		NITROGEN		PEPSIN: METT TUBE VALUE
					Total	Free	Total	Soluble	
	ml.	*gm./100 ml.*	*ml./N/10 per 100 ml.*	*ml./N/10 per 100 ml.*	*mg./gm. dry solids*		*solids mg./gm.*		
	Period 1 — June 16–19 (Peeled wheat bread)								
1	247	7.90	95.8	83.4	559	147.3	27.02	12.89	19.3
2	226	8.49	103.0	90.0	557	108.4	26.68	13.72	20.2
3	192	6.77	68.9	57.6	538	152.7	26.88	12.73	14.1
4	277	7.36	88.4	76.0	529	132.4	26.94	13.36	11.9
5	235	7.80	80.7	68.2	568	151.7	25.18	11.48	11.9
6	221	12.48	80.4	56.3	584	131.0	27.26	12.56	10.7
Av.	233	8.46	86.2	71.9	556	137.3	26.66	12.79	14.7
	Period 2 — June 24–26 (Peeled wheat bread after ingestion of Ca pantothenate)								
1	259	6.49	95.3	86.3	493	135.3	26.10	14.07	19.2
2	245	9.07	99.7	86.0	566	106.1	25.44	14.03	20.8
3	169	7.51	81.9	70.0	561	145.6	26.10	12.45	17.2
4	276	7.41	79.4	63.0	576	144.1	27.46	14.60	15.4
5	252	6.34	68.4	58.7	560	166.1	25.74	13.42	10.7
6	233	12.05	71.8	49.1	581	144.4	28.07	14.72	9.7
Av.	239	8.14	82.1	68.8	556	140.3	26.48	13.88	15.5
	Period 3 — July 1–3 (Peeled wheat bread with high-vitamin yeast)								
1	270	5.77	99.1	87.8	518	133.4	28.02	14.43	18.1
2	173	7.43	101.5	87.8	533	120.0	30.24	16.37	22.8
3	197	8.03	86.3	69.7	549	154.1	26.73	12.55	16.4
4	279	6.92	77.9	65.8	563	148.8	27.06	14.69	11.3
5	207	8.04	80.2	64.8	567	175.4	27.40	12.50	14.2
6	220	13.83	80.1	55.9	542	138.4	27.30	13.52	10.8
Av.	224	8.33	87.5	71.9	542	145.0	27.79	14.21	15.9
	Period 4 — July 8–10 (High-extraction white bread)								
1	267	5.83	96.2	86.7	604	124.0	30.6	14.99	15.1
2	240	9.88	99.2	86.3	616	90.7	24.7	15.97	15.6
3	139	7.04	60.6	51.4	624	135.1	25.7	13.40	11.1
4	237	11.97	64.2	53.9	617	130.9	24.1	14.85	7.5
5	165	12.13	67.6	55.8	602	162.0	23.5	12.24	9.1
6	226	17.75	70.7	57.6	628	121.7	23.7	15.45	7.8
Av.	212	10.76	76.6	65.3	615	127.4	25.4	14.48	11.0
	Period 5 — July 15–17 (Ordinary white bread)								
1	203	7.19	88.4	79.4	646	127.4	25.1	18.4	15.8
2	236	13.22	89.0	78.5	623	94.2	23.6	16.2	14.5
3	144	10.37	64.9	54.2[1]	650	145.6	23.6	13.8	11.4
4	163	13.30	63.5	50.1	658	148.7	24.5	15.1	8.4
5	117	14.23	65.6	49.9	612	163.2	22.7	15.9	9.5
6	211	18.74	70.3	53.2	673	121.0	23.2	14.4	7.7
Av.	179	12.95	73.6	61.3	643	137.0	23.3	15.7	11.2

[1] Average of only 2 days.

When the daily results were examined for the first three periods comprising sixty individual test meals — forty-two on peeled-wheat bread, and eighteen on the same baked with high-vitamin yeast and differing only slightly in composition from the former — the values showed a perfectly random distribution not affected by any systematic error. This fact bears out the consistency of the results for each subject in the average analytical values of table 1. The close agreement for total solids per 100 ml. of stomach contents among the three average values in these periods, and among the average values for free acidity and pepsin in the stomach contents, is evidence not only that the subjects were performing consistently, but also that the error of determination was satisfactorily low and uniform. The agreement, also, in all these average values between the results from the two white breads is as good as could be expected in view of their differences in composition (table 2).

TABLE 2

Analysis of breads for reducing substances and nitrogen.

BREADS	REDUCING SUBSTANCE		NITROGEN	
	Total	Free	Total	Soluble
	mgm. per gm. dry wt.		*mgm. per gm. dry wt.*	
Peeled whole wheat bread	638	71.5	26.81	2.52
Peeled whole wheat bread (Period 2)	622	69.5	27.07	2.73
Peeled whole wheat bread (baked with high-vitamin yeast)	637	70.7	27.07	2.76
High extraction white bread	685	43.8	24.32	2.36
Ordinary white bread	667	52.4	24.40	2.20

The values which reflect the speed of digestion, however, are contained under the headings "Reducing Substance" and "Nitrogen", and consistency among them is the resultant of composition of the food material as this may influence digestibility, as well as performance of the gastric glands. Hence, we do not find the figures running parallel in different columns so closely as they do under free acidity and pepsin (table 1). Nevertheless, it is perfectly evident that high acidity and

high pepsin in the first two periods on the same bread were associated with more soluble nitrogen than were low acidity and low pepsin; and conversely, that high acidity tended to retard, and low acidity to favor, free sugar (reducing substance) formation from starch.

These values, however, are subject to two corrections: (1) variations in the time of sampling, and (2) the occurrence of soluble nitrogen and free reducing substance in the breads as eaten. The variations in time have already been explained. In some periods they were large enough to affect considerably the amount of digestion from the time the food was eaten until the drawn sample was boiled to stop the enzyme action. Whether the taking of 16 mg.[4] of calcium pantothenate several times before eating the peeled-wheat bread (see below) produced any significant change in rate of digestion as compared with the same bread eaten alone could not be established certainly without making these corrections.

For example, the difference between the average values for soluble nitrogen in periods 1 and 2 (table 1) is 1.09 mg. per gram of solids in the stomach contents, or about 8.5%. Applying the statistical analysis of Fisher ('36) for the difference between two means to the two sets of individual determinations — eighteen for each period — the probability (p) that this difference is due to chance, is less than .01, i.e., less than 1 in 100. It must therefore be due to the fact that calcium pantothenate increased the speed of digestion; there is no other factor to which it could be ascribed. However, this analysis is based on the uncorrected figures. Would the difference be increased by using corrected values?

In table 3 are shown the effects of making the two corrections on the original values for 3 days of period 2. In arriving at the corrections for time, the average values obtained for elapsed times (from 60 to 70 minutes) with the peeled-wheat bread alone between completion of the meal and completion of sample were plotted and were found to fall in a straight line.

[4] This dose was chosen because an equivalent amount per kilogram of body weight had proven effective in the deficient dogs (Bly, Heggeness and Nasset, '43).

TABLE 3

Sample corrections of individual values for time and preformed substances in bread[1] *(all values in mg./gm. solids).*

SOLUBLE N				FREE SUGAR			
Original crude value	Corrected for time	Corrected for sol. N in bread	Correct value	Original crude value	Corrected for time	Corrected for free sugar in bread	Correct value
16.14	...	2.73	13.41	174.7	..	69.5	105.2
14.85	—0.56	2.73	11.56	147.8	—3.2	69.5	75.1
11.22	...	2.73	8.49	152.2	—3.2	69.5	79.5
15.46	...	2.73	12.73	171.7	—6.4	69.5	95.8
12.86	+0.28	2.73	10.41	142.4	+3.2	69.5	76.1
13.87	—0.84	2.73	10.25	109.8	+3.2	69.5	43.5
13.45	—0.28	2.73	10.44	172.1	..	69.5	102.6
11.72	+0.28	2.73	8.71	141.1	..	69.5	71.6
12.19	—0.28	2.73	9.19	155.5	—3.2	69.5	82.8

[1] The days corrected for are chosen at random to show the range of original values and of corrections for time.

From this graph the corrections per minute were derived. With one of the white breads the corrections for time were not possible for two reasons: (1) on account of the higher relative content of gluten the stomach contents were inclined to form clumps, thus obstructing the sampling tube more and thereby producing greater variations in the sampling time which were not due to mere delay in getting started; and (2) there were some obvious errors in recording the times for this period. It turns out, however, that the difference between the mean value for this period and the other white bread period, as well as between periods 5 and 1 (peeled-wheat bread eaten alone) was great enough to be just as significant without this correction as with it (judging by the effect of such correction in period 4).

Time corrections, it will be noted (table 3), are sometimes positive but more often negative, meaning that the time of drawing the sample was longer than 60 minutes from the time the meal was finished more times than it was less than 60. The corrections for the soluble N and free sugar were based on the average of several analyses and are constant. The re-

sult is that the difference between the two means discussed is increased from 1.09 mg. to 1.33 mg. per gram sample.

Applying statistics to all the individual corrected values the results are as shown in table 4. The percentage difference between the two means for soluble N in periods 1 and 2 is now 14.9% instead of 8.5% but the probability value (p) is not changed (Fisher's table stops at .01).

TABLE 4

Summary of digestion data for the several breads and signicance of difference between means.

PERIODS COMPARED	BREADS COMPARED	SOLUBLE N				FREE REDUCING SUBSTANCE			
		Corrected means compared		% difference	p	Corrected means compared		% difference	p
		mg./gm. solids				*mg./gm. solids*			
1 and 2	P.W.[1] bread and same + Ca pant.	8.91	10.24	14.9	.01	64.6	71.1	10.0	.01
1 and 3	P.W.[1] bread and same with hi-vitamin yeast	8.91	10.28	15.3	.01	64.6	74.1	14.7	.01
1 and 4	P.W.[1] bread and high-extraction white bread with hi-vitamin yeast	8.91	10.93	22.5	.01	64.6	78.3	21.2	.01
1 and 5	P.W.[1] bread and ordinary white bread	8.91	13.10	45.9	.01	64.6	79.3	22.7	.01
4 and 5	High-extraction and ordinary white breads	10.93	13.10	19.8	.01	78.3	79.3	1.3	not sig.

[1] P.W. for "peeled wheat".

The comparisons between amounts of digestion with peeled-wheat bread and the same eaten with calcium pantothenate, on the one hand, and peeled-wheat bread and the same baked with high-vitamin yeast on the other, in periods 2 and 3, show practically no difference (10.24 and 10.28 mg. per gram solids (table 4)). This means the advantage gained by eating peeled-wheat bread baked with high-vitamin yeast is matched by eat-

ing the same bread baked with ordinary yeast after taking calcium pantothenate twice before the meal.

	POIL YEAST (DRY BASIS) μg./gm.	HIGH-VITAMIN YEAST (DRY BASIS) μg./gm.
Thiamine	128	700
Riboflavin	40	65
Nicotinic acid	300	340
Pyridoxine	20	40
Pantothenic acid	94	100 to 150 (mean 125)

Vitamin values for the yeasts [5] are given here based on the latest analyses. They do not necessarily represent the true values for the ordinary bakers' yeast and the high-vitamin yeast used in baking the two varieties of peeled-wheat bread tested in this investigation, because different batches of both yeasts have shown appreciable variations. They do serve, however, to give an approximate idea of the amount of extra vitamins obtainable from eating 150 gm. of the high-vitamin yeast bread as compared with the peeled-wheat bread baked with ordinary yeast. It is not likely that single selected batches of the two yeasts would present greater differences. From the figures for pantothenic acid given above, therefore, it may be calculated that the person eating 150 mg. of high-vitamin peeled-wheat bread would obtain 0.45 mg. more of this vitamin than he would when eating the same bread baked with ordinary yeast.

From determinations made in this laboratory (Sealock and Livermore, '43) the peeled-wheat bread baked with ordinary yeast contains 5.2 mg. of pantothenic acid per gram or 0.78 mg. in 150 gm. If we may assume that pantothenic acid is the only one of the B-factors affecting rates of digestion (Russell and Nasset, '41), then it appears that the addition of less than 1 mg. of this vitamin in the natural form in the bread, produces effects on digestion of protein and carbohydrates equal to or greater than the effects of over 15 times as much of the synthetic calcium salt taken as an "appetizer" an hour before the meal (first two comparisons in table 5).

[5] The authors are under special obligation to Dr. Charles N. Frey, Director of the Fleischmann Laboratories, for making the analyses available.

TABLE 5

Comparison of digestibilities of several breads. Soluble N and free sugar formed in 1 hour as percentages of insoluble N and total hydrolyzable carbohydrate in breads.

BREADS COMPARED	PERIODS	SOLUBLE N'S AS % OF TOTAL INSOLUBLE N IN BREAD		DIFFERENCE IN RATE %	FREE SUGAR AS % OF TOTAL HYDROLYZABLE CARBOHYDRATE		DIFFERENCE IN RATE %
Peeled wheat bread and same eaten after Ca pantothenate	1 & 2	36.6	42.1	15.0	11.8	12.9	9.3
Peeled wheat bread and same baked with high-vitamin yeast	1 & 3	36.6	42.3	15.6	11.8	13.1	11.0
Peeled wheat and high extraction white bread	1 & 4	36.6	49.8	36.1	11.8	12.2	3.4
Peeled wheat bread baked with high-vitamin yeast and high extraction white bread	3 & 4	42.3	49.8	17.7	13.1	12.2	—6.9 [1]
Peeled wheat bread and ordinary white bread	1 & 5	36.6	59.0 [2]	61.2	11.8	13.1	11.0
Peeled wheat bread baked with high-vitamin yeast and ordinary white bread	3 & 5	42.3	59.0	39.5	13.1	13.1	0.0
High extraction white bread and ordinary white bread	4 & 5	49.8	59.0	18.5	12.2	13.1	7.4

[1] With this single exception the rate of digestion of the second member of each pair is higher.

[2] The more rapid digestion of protein is due to higher relative gluten content in the ordinary white bread, and to the dry milk solids.

This is a surprising and gratifying result, but one must not conclude too hastily that no other factor in the high-vitamin yeast has contributed a share to the more favorable effect. There is, for example, in the work of Bly, Heggeness and Nasset ('43) some evidence that small amounts of inositol produce in dogs a measurable acceleration (+14%) in the digestion rate of the carbohydrate in peeled-wheat bread. Another view, which equally commends itself, is that when a small addition of a vitamin proves adequate to produce a full digestion rate any addition above this adequate level is ineffective. It is clearly possible that 1 mg. represents the adequate dose for pantothenic acid.

The other comparisons in table 4 will be self-explanatory. It is of interest that, with respect to the amount of soluble N formed in 1 hour, the high extraction white bread baked with high-vitamin yeast excels the peeled-wheat bread more than does the peeled-wheat bread baked with this yeast. Probably the finer texture of the white bread and the higher relative gluten content make the difference. The standard or "ordinary" white bread is highest in the percentage of soluble N formed. The percentage difference between it and the peeled-wheat bread is twice that between the high extraction white bread and the peeled-wheat bread (table 4). This probably is due to the slower rate of digestion of proteins contained in the aleurone cells and other more fibrous portions of the grain, in the high extraction bread.

None of the comparisons on the formation of free sugar from values corrected in the same manner as those for soluble N reveals differences as large as the corresponding values for digestion of protein. In general, however, they fall in the same order. Only the difference between high extraction white bread and the ordinary white bread is not significant.

In preparation of the necessary values for comparison of rates of digestion, the amounts of insoluble N (protein) and total hydrolyzable carbohydrate present in the several breads when they were eaten have been taken into account. These quantities are derived from the data in table 2, and when the corrected values for soluble N and free sugar (table 4) are expressed as percentages of such quantities, we have the relative rates as between the several pairs of breads (table 5).

For the same comparisons (table 4) the differences between the rates per 100 parts of protein per hour (table 5) are always of the same order of magnitude as the corresponding differences in absolute amounts per hour (table 4). This might mean, of course, that the perecentage of protein in the several breads was equal, but from table 2 it is evident that the whole wheat bread contains nearly 3% more protein [6] than the two

[6] It is well-known that a small portion of the insoluble N in whole wheat does not represent true protein. (See Winton and Winton, Structure and Composition of Foods, Vol. I, Cereals, 1932, p. 211, et seq.).

white breads which happen to be nearly equal, and some of it is less readily available for digestion. Not knowing what this correction is, we must conclude from the figures as they stand that a larger percentage of the protein in the white breads than in the whole wheat breads is rendered soluble in the same length of time by gastric digestion.

This is not true of the amylolytic digestion of starch by salivary amylase in the stomach. For example, the rate of digestion of high-vitamin peeled-wheat bread by this enzyme is 11% more rapid than that of peeled-wheat bread with ordinary yeast, and the ordinary white breads exceed peeled-wheat bread by exactly the same percentage difference. It follows, of course, that the high-vitamin peeled-wheat breads and ordinary white breads are equal as shown. It is interesting that the whole wheat bread baked with high-vitamin yeast exceeds by a small percentage the high extraction white bread baked with a smaller amount of the high-vitamin yeast.

DISCUSSION

The most significant result among those summarized in table 5 is that a small extra amount of pantothenic acid (possibly aided by some other B-factor) contained in high-vitamin yeast has a favorable effect on digestion at least as great as much larger amounts of the synthetic calcium salt of this acid taken in at least two doses: one, an hour before the test meal, and the other 8 to 10 hours before.[7]

Next in importance is the finding that, with respect to the digestion of starch contained in breads, the use of high-vitamin yeast seems to bring the digestibility of whole wheat bread up to that of ordinary, low extraction white bread. This appears to offer a clue to the preparation of a "successful" whole

[7] It should be explained that the calcium pantothenate was administered in this manner to avoid adsorption of the compound on barium carbonate which was being used on adjacent days to render visible by x-ray examination any effects the various B-factors might have on gastric motility. There appeared to be good evidence for nullification of such effects by barium compounds. These experiments have been discontinued pending the development of a more suitable opaque medium.

wheat bread, but the improvement as yet applies only to digestion of "hydrolyzable carbohydrate". Possibly the addition of non-fat milk solids would increase protein digestion (in the stomach) also to the level of that of ordinary white bread. Further experiments will be necessary to clarify this and some other questions involved in the production of a wholly acceptable whole (98%) wheat bread.

It is of special interest that in the gastric digests of the whole wheat bread the average pepsin content was approximately 40% greater (Mett tube measurement) than in those of the two white breads. The peptogenic value of whole wheat bread, therefore, is not to blame for lower rates of protein digestion, and such error as results from the small amount of (soluble) nitrogen in the pepsin does not invalidate but rather gives emphasis to the slower rate of gastric digestion of the whole wheat product. The vital economic value of whole wheat as food for man is not touched by these facts, for the over-all digestion is sufficiently high to produce a large net saving not only of protein but also of calories from the wheat for human consumption (Murlin, Marshall and Kochakian, '41).

From this study it appears plausible that the slower rate of digestion of whole wheat in the human stomach as compared with white breads is responsible at least in part for the relative unacceptability of the former by the general public.

SUMMARY AND CONCLUSIONS

Peeled-wheat bread, which is made from flour containing all of the wheat kernel except the outer epidermis weighing less than 2%, has been studied in comparison with several other breads in experiments on gastric digestion in six human subjects. Samples drawn from the stomach by means of the Rehfuss tube 1 hour after eating were analyzed for total and free acidity, total solids, pepsin, total and free reducing substance, and total and soluble nitrogen. Corrections for time lost in sampling and for free reducing substance and soluble nitrogen in the breads made possible the calculation of rates of carbohydrate and protein gastric digestion.

The peeled-wheat bread baked with high-vitamin yeast undergoes gastric proteolytic digestion 15% faster than when the bread is baked with ordinary bakers' yeast; the free sugar formation under the amylolytic action of saliva is 11% faster. Calcium pantothenate in 16 mg. doses taken at least twice before the meal (8 to 10 hours and 1 hour) accelerates the two digestion rates to about the same extent. Pantothenic acid in the test meal of high-vitamin yeast bread exceeded that of the same bread baked with ordinary yeast by only 0.45 mg. If this is the only vitamin B-factor affecting digestion rates, it appears that a relatively small amount in the bread is as effective as a much larger amount taken before the meal.

A "high extraction" white bread containing 3.5% milk solids, based on the flour, and made with high-vitamin yeast, showed a digestion rate for protein and carbohydrate 36% and 3%, respectively, faster than that for the peeled-wheat bread. A white bread made of "straight grade" flour representing an extraction, according to the manufacturer, of "about 72% of the entire wheat berry" and containing 2.5% non-fat milk solids based on the flour showed digestion rates 61% and 11%, respectively, faster than those for the peeled-wheat bread. This white bread, however, was digested only 39% faster than the peeled-wheat bread baked with high-vitamin yeasts with respect to protein, and not at all faster as regards the carbohydrate.

The shortcoming of whole wheat bread does not lie in a lesser peptogenic effect, for the pepsin content in the gastric digests was 40% higher than in those from the white breads.

LITERATURE CITED

BLY, C. G., F. W. HEGGENESS AND E. S. NASSET 1943 The effects of pantothenic acid and inositol added to whole wheat bread on evacuation time, digestion and absorption in the upper G. I. tract of dogs. J. Nutrition, vol. 26, p. 161.

FISHER, R. A. 1936 Statistical methods for research workers. Oliver and Boyd, London.

MURLIN, J. R., M. E. MARSHALL AND C. D. KOCHAKIAN 1941 Digestibility and biological value of whole wheat breads as compared with white bread. J. Nutrition, vol. 22, p. 573.

RUSSELL, R. A., AND E. S. NASSET 1941 The effect of various vitamin supplements and of whole yeast on the digestion and absorption of the carbohydrate of a complete diet. J. Nutrition, vol. 22, p. 287.

SEALOCK, R. R., D. H. BASINSKI AND J. R. MURLIN 1941 Apparent digestibility of carbohydrates, fats, and "indigestible residue" in whole wheat and white breads. J. Nutrition, vol. 22, p. 589.

SEALOCK, R. R., AND A. H. LIVERMORE 1943 The vitamin B-complex of peeled-wheat bread. J. Nutrition, vol. 25, p. 265.

THE EFFECT OF SODIUM CHLORIDE UPON THE DISPOSITION OF INJECTED GLUCOSE IN A STRAIN OF RATS[1]

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ONE FIGURE

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That sodium chloride is related in some way to the metabolism of glucose is suggested by a number of reports in the literature. The onset of diabetes seems to be followed by an increased excretion of sodium chloride with a consequent depletion of this electrolyte in the body fluids. Adlersberg and Wachstein ('37) observed that both sodium and chloride levels fall in the blood and tissues following pancreatectomy in the dog. This is said to be due to an increased urinary loss of sodium chloride. Following the withdrawal of insulin from human diabetics a large amount of base is lost in the urine and the quantity combined with ketones is much less than the quantity sacrificed with chloride (Peters, Kydd, Eisenman and Hald, '33). Sodium chloride, given in large daily doses, has been reported to have a favorable effect upon the utiliza-

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tion of glucose by human diabetics (McQuarrie, Thompson and Anderson, '36; MacLean, '35). This interesting observation, however, could not be confirmed by Strouse, Buel, Kay and Drury ('41).

A diet rich in sodium chloride, when fed to experimental animals, enhances the deposition of glycogen in the liver (Crabtree and Longwell, '36), increases the fall in blood sugar following insulin administration (Adlersberg and Wachstein, '37; Lewis and Longwell, '41), and improves the glucose tolerance of pancreatectomized dogs (Adlersberg and Wachstein, '37). The tolerance of the pancreatectomized rat to intraperitoneally administered glucose is improved when sodium chloride is given along with the glucose (Orten and Devlin, '40).

The present experiments were designed to study the effect of sodium chloride upon the disposition of intraperitoneally administered glucose in the rat. During the course of the investigation it was discovered that a high percentage of rats of the stock colony had a low tolerance to glucose (Orten and Devlin, '40).

The low tolerance to glucose of the Yale strain of rats has been the subject of a series of investigations (Cole and Harned, '38; Cole, Harned and Keeler, '41). These authors have presented evidence in support of the view that the phenomenon is related to an hyperpituitary function in this strain (Harned and Cole, '39). Furthermore, the adrenals of male rats of the Yale strain are significantly heavier than adrenals of male Wistar rats (Cole and Harned, '42). Carbohydrate balance studies indicate that an impairment in the mechanism of glycogenesis or glycogenolysis is the basis for the low tolerance to glucose (Orten and Sayers, '42). Connected with this observation is the fact that adrenal-medullectomy is followed by a normal tolerance to glucose in these animals (unpublished observations made in this laboratory).

The action of sodium chloride in improving the glucose tolerance of the Yale strain of rats has been investigated in the present study from the standpoint of its effect upon absorp-

tion, excretion, storage and transformation of the administered glucose. The improvement is shown to be the result of an increased storage of glucose as glycogen in the salt-treated animals.

EXPERIMENTAL

The animals used in this study were healthy, adult female rats of the Connecticut Agricultural Experiment Station ("Yale") and Wistar strains, weighing from 200 to 350 gm. Special care was taken to lessen variation in stored carbohydrate due to diversity in food consumption. Three days prior to glucose administration the stock colony ration,[3] which had been given ad libitum up to this time, was fed to the animals at 10:00 A.M. daily in an amount equal to 10 gm. per 250 gm. of body weight $\pm$ 1 gm. for each 50 gm. deviation in body weight. The animals were fasted 16 to 18 hours before experimentation.

A fasting blood sample was withdrawn from a tail vein at the beginning of the experimental period. This was followed by the intraperitoneal injection of a standard dose of glucose (350 mg. per 100 gm. body weight). Two types of glucose solutions were used, 8.75% glucose in water and 8.75% glucose in 0.85% NaCl. The solutions were warmed to 38°C. and injected accurately, being delivered from a 25 ml. pipette graduated in 0.1 ml. The animals were then placed in metabolism cages, and the urine collected during a 5-hour period. At the end of this time another sample of blood was withdrawn from a tail vein and nembutal (3 mg. per 100 gm. body weight) was injected intraperitoneally. In rapid succession the abdomen was opened, the peritoneal fluid quantitatively removed, the urinary bladder punctured, drained and washed, and the liver separated from the carcass and both plunged into a mixture of ether and solid carbon-dioxide. Uninjected controls received a similar treatment.

[3] Stock colony ration: "Calf builder" meal, 91% (Larrowe Milling Co., Detroit); wheat germ, 3%; dried brewers' yeast, 3%; and U.S.P. cod liver oil, 3%.

Methods

Glucose was determined in urine, peritoneal fluid, blood, tissue and glycogen hydrolysates by the method of Hagedorn and Jensen ('23) or the macro-modification of Hanes ('29). The question of non-sugar reducing substances was carefully investigated in all fluids with the exception of urine where the total reducing substances were too small to significantly affect the results of this investigation. Yeast fermentation studies on a number of blood filtrates and peritoneal fluids showed a relatively constant, small amount of non-sugar reducing substances to be present. The averages of these determinations were used to correct all blood and peritoneal fluid sugar values. Non-sugar reducing substances amounted to 14 mg. per 100 ml. of blood and 3.7 mg. per 100 mg. of unabsorbed glucose in the peritoneal fluid. Each individual free sugar and total carbohydrate determination on tissue (liver and carcass) was calculated in terms of fermentable sugar in the filtrate. Glycogen was calculated in terms of the fermentable sugar in the acid hydrolysates of this substance. The sum, tissue free sugar plus tissue glycogen, was practically equal in most instances to tissue total carbohydrate (see table 2 and fig. 1).

Preparation of tissues for analysis

The liver was crushed to a powder in the frozen state by means of a contusion mortar (Graeser, Ginsberg and Friedemann, '34). The powder was thoroughly mixed to insure homogeneity. The rest of the carcass, skin and bones was first broken into small pieces and then minced twice in a grinder. Both the tissue and the grinder were left in contact with "dry ice" during the mincing process. The minced tissue was thoroughly mixed to insure homogeneity and aliquots were taken for the analyses to be described. Determinations on duplicate samples invariably checked. This procedure was adopted after a number of glycogen analyses had been made on individual muscles (gastrocnemii), frozen in situ. The glycogen content of the right gastrocnemius was not in-

variably the same as that of the left. We have been forced to agree with the following statement of Chambers and Barker ('40) concerning analyses on small muscle samples: "A principal objection to these is the practical impossibility of judging the glycogen (or other carbohydrate) content of the entire body musculature from one or two isolated muscle samples."

TABLE 1

Methods of carbohydrate analyses.

ANALYSIS	TISSUE	AMOUNT OF TISSUE	PROTEIN PRECIPITATED BY ADDITION OF			AUTHORITY
			1 N H_2SO_4	10% $ZnSO_4$	2 N NaOH	
		gm.	*ml.*	*ml.*	*ml.*	
"Free" glucose	Liver	2	10.0	6.0	7.3	Blatherwick et al., ('35)
"Free" glucose	Carcass	20	50.0	30.0	34.5	Blatherwick et al., ('35)
Total carbohydrate	Liver	2	20.0	12.0	13.5	Cori and Cori ('33)
Total carbohydrate	Carcass	50	7.5[1]	6.0	5.6	Cori and Cori ('33)
			TISSUE DISSOLVED IN			
Glycogen	Liver	1	4 cc. 30% KOH			Good et al., ('33)
Glycogen	Carcass	20	40 cc. 30% KOH			Good et al., ('33)

[1] Amount present in 10 ml. aliquot of tissue hydrolysate.

Tissue analyses

Table 1 gives the amounts of tissues and reagents used in the various analyses, together with the references to the procedures used. In the liver, where the amount of tissue is limited, duplicate determinations for free sugar and total carbohydrate were made on the filtrate of single 2 gm. samples. Two separate 1 gm. samples of liver were used for glycogen determinations. In the carcass, four 20 gm. and one 50 gm.

aliquots were analyzed. Of the four 20 gm. samples, two were used for free sugar and two for glycogen. The 50 gm. sample used for total carbohydrate determination was hydrolyzed 4 hours in 300 ml. of 1 N H_2SO_4 and then diluted to 400 ml. volume. A pipette with an orifice 1.5 mm. in diameter was used to withdraw two 10 ml. aliquots of the fine suspension of tissue.

Glucose transformed

It is possible to calculate from the two separate sets of data the amount of injected glucose transformed to other substances during the course of the 5-hour period. The value represents the amount of injected glucose unaccounted for in the analyses. The following equations have been used to make the calculation: (1) Total carbohydrate of uninjected control, plus glucose absorbed and retained, minus total carbohydrate of injected animal equals the glucose transformed during the 5-hour period. (2) Glycogen plus free sugar of uninjected controls, plus glucose absorbed and retained, minus glycogen, plus free sugar of injected animal, equals glucose transformed during the 5-hour period. The values calculated from equation (1) agree closely with those calculated from equation (2) (see table 2 and fig.1). It is to be emphasized that the value for glucose transformed, as here defined, not only includes glucose oxidized, but also glucose converted to fat, pyruvic acid, lactic acid and other possible intermediates.

RESULTS

The present investigation is concerned with the effect of sodium chloride upon the characteristic low tolerance to glucose of the Yale strain of rats. The nature of this abnormality has been extensively reported elsewhere (Orten and Sayers, '42), but to facilitate an understanding of the action of NaCl the responses to glucose characteristic of a presumably normal strain (Wistar) have been included in table 2. Any change produced by this salt in the carbohydrate levels of the Yale strain can be compared to the already normal levels of the

TABLE 2
Disposition of glucose in Wistar, Yale and NaCl treated Yale rats.

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Disposition of glucose in Wistar, Yale and NaCl treated Yale rats.

DISTRIBUTION OF CARBOHYDRATE	YALE FASTING (6) [1]		WISTAR FASTING (6)	WISTAR GLUCOSE ALONE (9)		YALE GLUCOSE ALONE (10)		YALE GLUCOSE + NaCl (9)	
	Mean and S. D.[2]	S. R.[3]	Mean and S. D.	Mean and S. D.	S. R.	Mean and S. D.	S. R.	Mean and S. D.	S. R.[4]
Glucose absorbed mg./100 gm. rat				277 ± 35.6	0.0	277 ± 28.4	0.47	281 ± 26.0	0.40
Glucose excreted mg./100 gm. rat				3 ± 1.5	0.88	4 ± 5.3	2.58	9 ± 7.3	3.70
"True" blood glucose									
Initial mg./100 cc.	77 ± 4.7	5.81	66 ± 10.3						
Final mg./100 cc.				115 ± 25.0	5.19	219 ± 90.4	4.35	125 ± 42.9	0.89
Liver									
"Free" glucose mg./100 gm. rat	2.8 ± .36	4.65	2.1 ± .39	6.4 ± .93	3.89	8.5 ± 2.16	3.26	5.8 ± 2.72	0.88
Glycogen [5] mg./100 gm. rat	10.6 ± 4.65	3.15	5.4 ± 3.79	45.3 ± 21.57	2.37	30.7 ± 17.98	3.21	44.4 ± 8.58	0.18
Total carbohydrate mg./100 gm. rat	17.4 ± 6.72	2.98	10.3 ± 5.50	54.9 ± 19.12	1.96	43.2 ± 19.55	2.80	57.7 ± 13.94	0.49
Carcass									
"Free" glucose mg./100 gm. rat	22 ± 6.5	1.20	19 ± 6.6	42 ± 11.4	4.37	70 ± 24.5	4.40	41 ± 12.2	0.24
Glycogen mg./100 gm. rat	239 ± 68.2	0.58	253 ± 56.6	359 ± 60.8	6.70	234 ± 57.0	6.57	370 ± 68.3	0.52
Total carbohydrate mg./100 gm. rat	285 ± 34.0	1.27	263 ± 53.1	389 ± 48.7	2.26	346 ± 69.4	2.86	400 ± 45.0	0.73
Summary (Carcass and liver)									
Total carbohydrate data									
Total carbohydrate mg./100 gm. rat	302 ± 31.5	1.67	273 ± 55.0	444 ± 47.4	2.52	389 ± 84.7	3.20	458 ± 43.2	0.95
Total carbohydrate in excess of fasting level mg./100 gm. rat				171 ± 47.4	2.52	87 ± 84.7	3.20	156 ± 43.2	0.95
Injected glucose transformed [6] mg./100 gm. rat				103 ± 72.0	3.24	185 ± 86.2	3.28	112 ± 45.5	0.45
"Free" glucose and glycogen data									
"Free" glucose and glycogen, mg./100 gm. rat	274 ± 73.1	0.23	280 ± 60.7	453 ± 51.3	6.08	343 ± 65.2	5.69	461 ± 64.9	0.42
"Free" glucose and glycogen, in excess of fasting level mg./100 gm. rat				173 ± 51.3	5.74	69 ± 65.2	5.67	187 ± 64.9	0.73
Injected glucose transformed,[7] mg./100 gm. rat				101 ± 84.8	4.16	203 ± 68.6	5.15	81 ± 75.4	0.76

[1] Number of animals in parentheses.
[2] S. D. = standard deviation.
[3] S. R. = significance ratio (Sherman, '37) between means immediately to the right and left.
[4] Between Yale strain (glucose + NaCl) and Wistar strain (glucose alone).
[5] All carbohydrate values expressed in terms of glucose.
[6] Glucose absorbed minus glucose excreted minus total carbohydrate present in excess of fasting level.
[7] Glucose absorbed minus glucose excreted minus ["free" glucose + glycogen present in excess of fasting level.]

Wistar strain given glucose alone. From table 2 it is to be noted that there is no significant difference in the peritoneal absorption or renal excretion of the injected glucose in the two strains. In the uninjected controls, the Yale strain animals have significantly higher levels of blood sugar, liver free

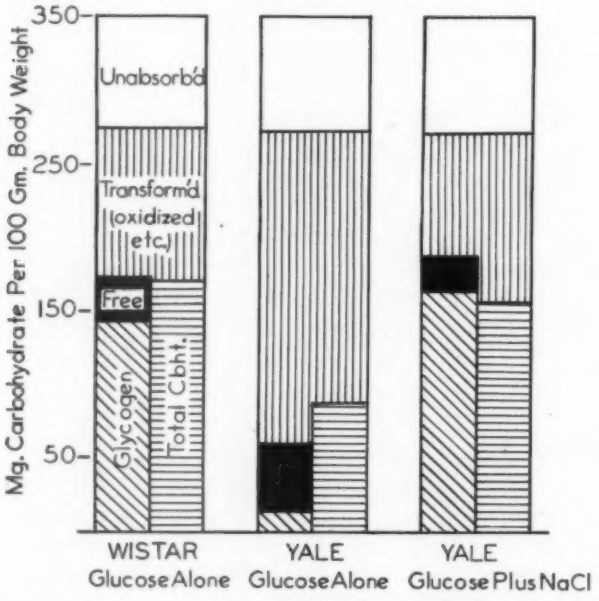

Fig. 1 Summary diagram showing effect of NaCl on the disposition of injected glucose in Yale strain of rats which have a low tolerance to glucose.

sugar, liver glycogen and liver total carbohydrate. No significant difference exists between the carcass values in the fasting state. In the injected animals, however, smaller amounts of glycogen in the liver and remaining carcass are to be found in the Yale strain, together with significantly larger amounts of "free" sugar.

From table 2 it can also be seen that the very slightly higher average level of glucose absorption in the sodium chloride treated animals is not significantly different from that of the

Yale and Wistar strains given glucose alone. The salt does exert a slight glycosuric action, but when the amount of glucose absorbed and retained is calculated it happens that this value is the same in all three groups of animals. When the NaCl is given in a concentration isotonic to the glucose, i. e., 1.58%, the absorption of the glucose is not affected, but a more marked glycosuria takes place whose influence upon the amount of glucose absorbed and retained makes this higher salt concentration unsuitable for a balance study (Sayers and Orten, '41).

Examination of table 2 shows that the fifth hour blood sugar of the "Yale" strain is considerably lowered when the animals are given NaCl with the administered glucose; in fact, the values are essentially normal when compared with those of the Wistar strain. This confirms previous reports from this laboratory (Orten and Devlin, '40), using 1.58% NaCl. It is to be noted that with the weaker concentration of salt a normal tolerance is obtained in a situation where the amount of glucose absorbed and retained is the same in both the "glucose-alone" and the "salt-treated" animals.

Significant differences in tissue carbohydrate levels between the NaCl-treated rats and the Yale strain animals given glucose alone are shown in table 2. In both the liver and carcass, the free sugar is decreased and the glycogen and total carbohydrate are increased in the salt-treated animals. When the significance ratios between the averages of the three groups — the "Wistar" animals given glucose alone, the "Yale" rats given glucose alone, and the "Yale" animals receiving sodium chloride — are examined, it can readily be seen that NaCl brings about the production of normal carbohydrate levels in the otherwise abnormal "Yale" strain rat. Calculation of glucose transformed (oxidized, etc.) shows that this value is reduced to a normal level in the "Yale" strain animals given NaCl. Apparently, the animals are enabled to store enough of the injected glucose to be commensurate with a normal tolerance curve.

All of the above facts are diagrammatically represented in figure 1.

DISCUSSION

The injection of a hypertonic solution of glucose into the peritoneal cavity is followed by the accumulation of electrolytes in this space at the expense of the blood and tissues (Darrow and Yannet, '35). In this connection it should be noted that the "Yale" strain animals, in contrast to those of the Wistar strain, present a picture of irritability, and even in some cases of muscular spasticity beginning with the second or third hour subsequent to the administration of glucose. Sodium chloride-treated rats of the "Yale" strain show none of these symptoms. Five hours after the administration of the glucose, the peritoneal cavity contained a volume of fluid twice as great as the amount injected. Not only was this true of the Wistar and "Yale" animals given glucose alone, but also of the animals given glucose dissolved in sodium chloride of either 1.58% or 0.85% concentration. Neither the strain difference nor the action of sodium chloride influenced the amount of fluid lost by the animal. A strain difference may exist in the amount of sodium chloride passing into the peritoneal cavity. No quantitative studies of electrolyte balance, necessary to settle this problem, have been made.

Concentrations of 0.85% and 1.58% sodium chloride are equally effective in improving the low tolerance to glucose of the "Yale" strain of rats. A factor to be considered in the higher concentration of salt is the loss of glucose by way of the kidney. That this is neither a necessary nor a primary effect of the salt is proven by the fact that 0.85% sodium chloride acts in a situation where the amount of glucose absorbed and retained is the same as in the "glucose alone" treated animals. McQuarrie, Thompson and Anderson ('36) have reported a decreased urinary loss of glucose in certain human diabetics under sodium chloride therapy.

Sodium chloride does not help the utilization of glucose by way of mechanisms involving its transformation (oxidation, etc.); in fact, by calculation (see table 2 and fig. 1) it appears

that less glucose is utilized by this means in the presence of salt. The accompanying reduction in muscular activity after sodium chloride treatment may account for this decrease.

The improved glucose tolerance of the "Yale" strain of rats given sodium chloride is definitely a consequence of the increase in the deposition of administered glucose as glycogen. We have no knowledge of the exact mechanism involved. Lewis and Longwell ('41) found the tolerance to glucose and the sensitivity to subcutaneously administered insulin to be greater in animals maintained on a high sodium chloride diet than in control animals. Their evidence suggests that storage changes rather than alterations in oxidative reactions brought about the observed results. This is in agreement with our findings. Adrenal demedullation as well as NaCl administration result in the production of a normal tolerance in the "Yale" strain of rats (unpublished data). It is possible that NaCl (1) decreases epinephrine secretion, (2) inhibits the glycolytic action of epinephrine, (3) counteracts vascular disturbances incident to hyper-secretion of epinephrine, or (4) operates independently of this hormone.

SUMMARY

The effect of sodium chloride on the disposition of intraperitoneally injected glucose has been studied in a strain of rats having a low tolerance to glucose.

Sodium chloride (0.85%) given with the glucose improves the tolerance of these rats, but has no significant effect upon the peritoneal absorption or renal excretion of the glucose.

The administration of sodium chloride with glucose favors the deposition of glycogen in both the liver and the rest of the carcass, simultaneously decreasing the "free" sugar in the blood and tissues. The transformation (oxidation, conversion to fat, etc.) of glucose also appears to be significantly decreased to a normal level.

It appears that sodium chloride improves the low tolerance to glucose of the "Yale" strain of rats primarily by increasing the storage of the administered glucose as glycogen.

LITERATURE CITED

ADLERSBERG, D., AND M. WACHSTEIN 1937 Pankreas und Kochsauzloffwechsel. Klin. Wchnschr., vol. 16, p. 85.

BLATHERWICK, N. R., P. J. BRADSHAW, M. E. EWING, H. W. LARSON AND S. D. SAWYER 1935 The determination of tissue carbohydrates. J. Biol. Chem., vol. 3, p. 537.

CHAMBERS, W. H., AND S. B. BARKER 1940 Carbohydrate Metabolism. Ann Rev. Biochem., vol. 9, p. 253.

COLE, V. V., AND B. K. HARNED 1938 Diabetic traits in a strain of rats. Endocrinology, vol. 23, p. 318.

———— 1942 Adrenal and pituary weights in rats with reduced glucose tolerance. Endocrinology, vol. 30, p. 146.

COLE, V. V., B. K. HARNED AND C. E. KEELER 1941 Inheritance of glucose tolerance. Endocrinology, vol. 28, p. 25.

CORI, C. F., AND G. T. CORI 1933 A comparison of total carbohydrate and glycogen content of mammalian muscle. J. Biol. Chem., vol. 100, p. 327.

CRABTREE, D. G., AND B. B. LONGWELL 1936 Effect of excessive dietary sodium chloride upon liver and muscle glycogen in the rat. Proc. Soc. Exp. Biol. and Med., vol. 34, p. 705.

DARROW, D. C., AND H. YANNET 1935 The changes in distribution of body water accompanying increase and decrease in extra-cellular electrolyte. J. Clin. Invest., vol. 14, p. 266.

GOOD, C. A., H. KRAMER AND M. SOMOGYI 1933 The determination of glycogen. J. Biol. Chem., vol. 100, p. 485.

GREASER, J. B., J. E. GINSBERG AND T. E. FRIEDMANN 1934 A method for the analysis of tissues. J. Biol. Chem., vol. 104, p. 149.

HAGEDORN, H. C., AND B. N. JENSEN 1923 Zur Mikrobestimmung des Blutzuckers Mittels Ferricyanid. Biochem. Z., vol. 135, p. 46.

HANES, C. S. 1929 An application of the method of Hagedorn and Jensen to the determination of larger quantities of reducing sugars. Biochem. J., vol. 23, p. 99.

HARNED, B. K., AND V. V. COLE 1939 Hyperfunction of anterior pituitary in a strain of rats. Endocrinology, vol. 25, p. 689.

LEWIS, R. C. JR., AND B. B. LONGWELL 1941 The effect of excessive dietary sodium chloride and potassium chloride on the carbohydrate metabolism of normal rats. Am. J. Physiol., vol. 133, p. 362.

MACLEAN, A. R. 1935 Observations on administration of sodium chloride in diabetes. Proc. Staff Meet., Mayo Clin., vol. 10, p. 321.

MCQUARRIE, I., W. H. THOMPSON AND J. A. ANDERSON 1936 Effect of excessive ingestion of sodium and potassium salts on carbohydrate metabolism and blood pressure in diabetic children. J. Nutrition, vol. 11, p. 77.

ORTEN, J. M., AND H. B. DEVLIN 1940 The effect of sodium chloride on the glucose tolerance of the diabetic rat. J. Biol. Chem., vol. 136, p. 461.

ORTEN, J. M., AND G. SAYERS 1942 A comparison of the disposition of injected glucose in two strains of rats. J. Biol. Chem., vol. 145, p. 123.

PETERS, J. P., D. M. KYDD, A. J. EISENMAN AND P. M. HALD 1933 The nature of diabetic acidosis. J. Clin. Invest., vol. 12, p. 377.

SAYERS, G., AND J. M. ORTEN 1941 Sodium chloride and peritoneal absorption and renal excretion of glucose in normal and diabetic rats. Proc. Soc. Exp. Biol. and Med., vol. 46, p. 287.

SHERMAN, H. C. 1937 Chemistry of Food and Nutrition, 5th edition, p. 602. Macmillan Co., New York.

STROUSE, S., F. BUEL, R. KAY AND D. DRURY 1941 Sodium and potassium salts in the treatment of experimental diabetes and diabetes mellitus. J. Nutrition, vol. 21, p. 599.

THE EFFECT OF VITAMIN D ON CALCIUM RETENTIONS [1]

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Although most foods contain little or no vitamin D, the presence of important amounts of this substance has been demonstrated (Sherman, '41) in egg yolk, whole milk and butter fat. According to Park ('39), a large proportion of children and adults must receive a moderate amount of vitamin D in their food. This moderate amount, notably inadequate for the growth needs of infants and children, may be adequate for the adult because of his decreased need. Nevertheless, Park suggests that under certain conditions it is advisable for adults to have some additional source of vitamin D. He states that among those adults who need additional vitamin D are: (1) "those deprived of the opportunity of obtaining the vitamin from the sun, and (2) all persons whose diet is lacking in milk or is generally poor."

Sherman ('41) points out that although there is little evidence as to whether or not adults need vitamin D, it has been shown that vitamin D does not lower the minimum need for calcium. Jeans and Stearns ('39) state that observations on adults indicate, as for children, that the ingestion of vitamin D in no way lessens the requirement for calcium.

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Jeans and Stearns ('39) also state that the requirements for vitamin D for the adult who has ample intakes of calcium and phosphorus and a diet otherwise adequate, may be defined as the amount needed for maintenance of bony and dental structures during adult life, and that the most useful criterion of the vitamin D requirement is provided by studies of the utilization of calcium and phosphorus. They further suggest that "because the quantity of phosphorus retained varies with the nitrogen as well as the calcium retention, the quantity of calcium retention may be used as the most convenient guide."

In comparing the calcium, phosphorus and nitrogen retentions of a group of 124 young women who were on their customary self-chosen diets, with the corresponding retentions of a group of nine women who were on well-selected basal diets supplemented daily by 250, 500, and 750 cc. of milk, respectively, at different intervals (McKay et al., '42), it was noted that at corresponding intakes, the women on the controlled diets retained more calcium than those on their customary self-chosen diets. This improved retention was especially noticeable for the subjects who were ingesting less than the recommended allowance of 0.8 gm. calcium daily.

EXPERIMENTAL PROCEDURE

The study here reported is concerned with the possible effects of vitamin D upon retention of calcium when women were using well-selected diets. For the purpose of this investigation, six of the young women who had been subjects in the study of the effect of diet upon retention of calcium continued as subjects in further tests.

The six subjects had previously been on the basal diets supplemented daily by 250, 500, and 750 cc. of milk, respectively, for three 10-day periods, each observation period being preceded by a 15-day foreperiod. Foods included in the basal diet were similar in kind and amount for each subject (table 1). To provide for varying calorie needs, certain foods of negligible mineral content, such as butter, mayonnaise, French dressing, jelly, cookies, white bread and macaroni, were used ad libitum by the subjects.

Upon completion of the last of three observation periods, at which time the diet was supplemented by 750 cc. of milk, the subjects were returned to the basal diet supplemented by 250 cc. of milk. They continued on this dietary regimen until the calcium retentions were approximately the same as they had been during the previous periods when similar diets supplemented by 250 cc. of milk had been used.

At this point, to test the possible effect of supplemental vitamin D upon retentions, each subject was given daily approximately 500 I.U. of vitamin D.[2] At the conclusion of a 15-day

TABLE 1

Basal diet used during each 5-day period.

FOOD	AMT.	FOOD	AMT.	FOOD	AMT.
	gm.		*gm.*		*gm.*
Beef	400	Orange juice	200	String beans	70
Bacon	20	Grapefruit	100	Beets	70
Salmon	90	Tomato juice	200	Cabbage	100
Egg	150	Tomato	100	Carrots	100
Whole wheat bread	375	Banana	100	Lettuce	100
		Canned peaches	100	Peas	70
		Canned pineapple	80	Potatoes	400
				Potato sweet	100

foreperiod, there were two consecutive 5-day collection periods. This routine was followed for milk intake levels of 250, 500, and 750 cc., respectively. One subject was obliged to drop out of the study at the end of the first vitamin D period. This left a group of five women for the two remaining vitamin D studies.

Methods of collection, sampling, and analysis were similar to those described in the preceding study (McKay et al., '42). Six 10-day observation periods for each of the five subjects and two 10-day periods for the sixth subject are reported. Of the thirty-two 10-day studies, twelve were made in Kansas and twenty in Ohio.

RESULTS AND DISCUSSION

Table 2 summarizes data for the studies of women on corresponding calcium intakes with and without the vitamin D

[2] The vitamin D was in the form of viosterol capsules, provided by Mead, Johnson and Company, Evansville, Indiana.

supplement. At mean daily intakes of from 0.5 to 0.8 gm., amounts which were less than the daily allowance for women recommended by the Committee on Food and Nutrition (now Food and Nutrition Board) of the National Research Council ('41) the mean retention figure (0.013) for the six individuals who were using vitamin D in addition to the well-selected controlled diets was somewhat less than the corresponding figure (0.029) for those individuals who were having no vitamin D.

TABLE 2

Calcium metabolism data for forty-two studies of women on controlled diets (in grams).

SERIES I. NO ADDITIONAL VITAMIN D			SERIES II. ADDITIONAL VITAMIN D		
Nine individuals with 26 studies [1]			Six individuals with 16 studies		
No. of studies	Mean intakes	Mean balances	No. of studies	Mean intakes	Mean balances
Mean Daily Intake Range 0.500–0.799 gm.					
3	0.560	0.019	2	0.587	0.030
5	0.648	0.035	4	0.611	0.000
2	0.765	0.028	..	....	....
Mean	0.645	0.029		0.603	0.013
Mean Daily Intake Range 0.800–1.299 gm.					
3	0.809	— 0.061	3	0.886	0.082
2	0.940	0.067	2	0.918	0.112
3	1.061	0.133	1	1.094	0.103
5	1.147	0.104	3	1.167	0.103
3	1.264	0.076	1	1.200	0.158
Mean	1.064	0.069		1.029	0.104

[1] Compiled from data previously published (McKay et al., '42).

That this difference was not significant statistically was shown by application of Student's ('25) t test to the difference between the mean retentions adjusted to a mean intake ($P = 0.72$). The use of approximately 500 I.U. of vitamin D had no influence upon the calcium retentions of the six individuals who were ingesting from 0.500 to 0.799 gm. calcium daily. These data corroborate the conclusions of Sherman ('41) and of Jeans and Stearns ('39) that the use of vitamin D does not lower the minimum need for calcium.

TABLE 3

Calcium intakes and retentions or losses for five individuals (in grams).

BASAL DIET SUPPLEMENTED BY:	PERIOD	O. M. (KAN.)		B. W. (KAN.)		E. M. (OHIO)		F. H. (OHIO)		D. M. (OHIO)	
		Intake	*Retention*	*Intake*	*Retention*	*Intake*	*Retention*	*Intake*	*Retention*	*Intake*	*Retention*
250 cc. milk	I a										
1st 5 days		0.662	0.175	0.618	0.100	0.572	0.166	0.564	0.118	0.559	* 0.075
2nd 5 days		0.657	— 0.099	0.610	0.100	0.542	— 0.069	0.651	0.077	0.575	— 0.059
Mean		0.660	0.038	0.614	0.100	0.557	0.048	0.608	0.098	0.567	0.008
Plus 500 I.U. vit. D	I b										
1st 5 days		0.673	0.171	0.614	0.020	0.591	0.026	0.594	0.058	0.602	— 0.175
2nd 5 days		0.558	0.050	0.563	0.009	0.627	0.144	0.576	0.031	0.632	— 0.062
Mean		0.616	0.111	0.589	0.015	0.609	0.085	0.585	0.044	0.617	— 0.118
500 cc. milk	II a										
1st 5 days		1.026	— 0.083	1.027	0.171	0.819	0.168	0.817	0.092	0.786	— 0.132
2nd 5 days		0.830	— 0.012	0.874	0.048	0.774	0.071	0.824	— 0.119	0.831	— 0.155
Mean		0.928	— 0.048	0.951	0.110	0.796	0.120	0.820	— 0.014	0.808	— 0.144
Plus 500 I.U. vit. D	II b										
1st 5 days		0.934	— 0.058	0.903	0.143	0.886	0.053	0.921	0.116	0.913	— 0.015
2nd 5 days		0.893	0.196	0.857	0.118	0.878	0.098	0.923	0.079	0.878	0.096
Mean		0.914	0.127	0.880	0.131	0.882	0.076	0.922	0.098	0.896	0.040
750 cc. milk	III a										
1st 5 days		1.355	— 0.081	1.067	0.130	1.132	0.080	1.174	0.156	1.126	— 0.139
2nd 5 days		1.219	0.235	1.187	0.119	1.151	0.019	1.133	0.231	1.171	0.161
Mean		1.287	0.077	1.127	0.125	1.142	0.050	1.154	0.194	1.148	0.011
Plus 500 I.U. vit. D	III b										
1st 5 days		1.179	0.164	1.109	0.067	1.174	0.209	1.114	0.128	1.154	— 0.038
2nd 5 days		1.147	0.195	1.078	0.138	1.227	0.108	1.190	0.017	1.218	0.149
Mean		1.163	0.180	1.094	0.103	1.200	0.158	1.152	0.072	1.186	0.056

At intake levels of 0.8 gm. and above, evidence of slightly better utilization of calcium by the women on the basal diets supplemented by vitamin D was shown by the mean retention figure 0.104 as compared with 0.069. When these retention figures were adjusted to a mean intake and Student's t test applied to the difference, the result indicated some influence of the additional vitamin D but not enough to be significant ($P = 0.26$).

Although mean figures for calcium retentions showed no significant difference due to the use of vitamin D, there was considerable variation in individual reactions (table 3). For two women, B. W. and F. H., the total calcium retention for the three periods of vitamin D intake was less than when no supplemental vitamin D was used. Calcium utilization was therefore fully as efficient either with or without supplemental vitamin D. For D. M. and E. M., the evidence of improved retention with the use of vitamin D was inconclusive. The fifth subject, O. M., retained more calcium during each of the vitamin D periods than during corresponding periods when no vitamin D was given, total calcium retention without vitamin D being 0.067 gm. as compared to 0.418 gm. when vitamin D was used. When the Student's t test was applied, however, the difference between the two retentions was not found to be significant ($P = 0.15$).

It is sometimes assumed that the distribution of phosphorus and calcium excretion changes when vitamin D is given. No such change took place in the case of these young adults, the percentage of both calcium and phosphorus excreted in the urine being little changed as the result of the use of additional vitamin D.

SUMMARY AND CONCLUSIONS

Six young college women were given a well-selected basal diet supplemented daily by 250, 500, and 750 cc. of milk, respectively, for each of three collection periods of 10 days each. Subsequently, corresponding diets supplemented by approximately 500 I.U. of vitamin D were given to one of the subjects

for one 10-day period and to the other five subjects for three 10-day periods each. At mean daily calcium intake levels of less than 0.8 gm., no evidence was shown of improved calcium retention with vitamin D. At mean daily calcium intake levels of 0.8 gm. and above, there was some evidence of improved retention with vitamin D, but the improvement was so slight that it was not statistically significant.

Although for one of the five subjects the mean retention figures improved during each of the vitamin D periods, the difference between the two mean retention figures was not significant.

It is concluded that the addition of approximately 500 I.U. of vitamin D to diets which were well-selected had little influence upon the calcium retention, and that the well-selected diet provides for the vitamin D needs of the young adult.

LITERATURE CITED

COMMITTEE ON FOOD AND NUTRITION, NATIONAL RESEARCH COUNCIL 1941 Recommended allowances for the various dietary essentials. J. Am. Diet. Assoc., vol. 17, p. 565.

JEANS, P. C., AND G. STEARNS 1939 The human requirement of vitamin D. The Vitamins. The American Medical Association, Chicago, pp. 483, 499, 503.

MCKAY, H., M. B. PATTON, M. A. OHLSON, M. S. PITTMAN, R. M. LEVERTON, A. G. MARSH, G. STEARNS AND G. COX 1942 Calcium, phosphorus and nitrogen metabolism of young college women. J. Nutrition, vol. 24, p. 367.

PARK, E. A. 1939 The use of vitamin D preparations in the prevention and treatment of disease. The Vitamins. The American Medical Association, Chicago, p. 537.

SHERMAN, H. C. 1941 Chemistry of food and nutrition, sixth edition. The Macmillan Co., N. Y., pp. 440, 436.

STUDENT 1925 New tables for testing the significance of observations. Metron, vol. 5, p. 105.

THE EFFECTS OF PANTOTHENIC ACID AND INOSITOL ADDED TO WHOLE WHEAT BREAD ON EVACUATION TIME, DIGESTION AND ABSORPTION IN THE UPPER GASTRO-INTESTINAL TRACT OF DOGS [1]

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TWO FIGURES

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INTRODUCTION

In view of the recognized importance of the B-complex vitamins on gastrointestinal function, and on the basis of the favorable results on carbohydrate digestion and absorption with pantothenic acid indicated by Russell and Nasset ('41), it was felt that an expanded study should be made of the effect of this and other factors on the digestive tract. While McKibbin et al. ('40) and Schaefer et al. ('42) have mentioned alimentary canal changes in pantothenic acid deficiency, no report has included an extended study on the chronic effect of pantothenic acid deficiency on gastrointestinal function.

Our object was to study, in jejunostomized dogs, a chronic pantothenic acid deficiency with regard to gastrointestinal motility as well as carbohydrate and protein digestion and absorption.

EXPERIMENTAL

The procedures employed were similar to those outlined by Russell and Nasset ('41) and Lambooy and Nasset ('43). In adult mongrel dogs, Maydl jejunostomies were established

[1] Supported in part by a grant from the Continental Baking Company.

approximately 50 cm. caudad from the ligament of Treitz. At least 12 hours after last food (20 hours in the deficiency periods), the dogs were placed in a standing position in a stall and supported so as to provide the least possible discomfort. A two-lumened catheter with a rubber balloon on the end was introduced through the fistula well into the lumen of the gut. When the catheter was in place, the balloon was inflated through the small lumen with approximately 10 cc. of air, thus minimizing the loss of food past the catheter. Through the larger lumen of the catheter, the chyme, as it passed the enterostomy, was drawn by a negative pressure of 12 cm. of water into an appropriate receiving vessel.

After a pre-feeding collection period of at least ½ hour to make certain that the intestine was free of food, a weighed, analyzed test meal was fed. Collections of the chyme from the receiving vessel were made at approximately half-hour intervals, weighed, and the pH determined. The collections were then placed in a boiling water bath for 15 minutes to destroy all enzyme activity, pooled for each experiment, and stored at 5°C. until the analyses were made.

The following three methods were used to determine when the end of the experiment had been reached; in other words, to insure that the total emptying time required for complete passage of the test meal from the mouth to the catheter had elapsed: (1) the occurrence of a negative Benedict's qualitative sugar test on the collection; (2) the low weights of the half-hour collections; (3) the increase of pH occurring toward the last part of the experiment. The runs were continued for an additional hour or more to make certain that the intestine was empty. Since the emptying time and gastrointestinal motility must bear a fairly close, inverse relationship to each other, the former may be taken as a measure of the latter.

The basal diet which dog 1 received to establish a normal control consisted of water- and alcohol-extracted casein, 25%; alcohol-extracted sucrose, 41%; hydrogenated vegetable oil,[2]

[2] Primex.

18%; bone ash, 4%; dried brewers' yeast, 10%; and salts, 2%. The salts were those of the Phillip salt mixture as modified by Arnold and Elvehjem ('39), plus cobalt according to Frost et al. ('41).

The experimental diet consisted of a whole wheat bread made from flour prepared by the Earle flotation process. This bread was obtained fresh daily and fed moistened with water, about 400 gm. of bread being consumed. No difficulty was experienced in getting the dogs to eat the bread, and no dislike for it was ever evidenced, even after several months. The composition of the diet is given in table 1, the vitamin content having been determined by Sealock and Livermore ('43).

TABLE 1

Composition of experimental diet.

BREAD AS EATEN	VITAMIN CONTENT
	(μg./gm. fresh bread)
N 1.67% (protein 9.63%)	Thiamine — 2.73 (fermentation method)
	Riboflavin — 2.45 (rat growth)
Carbohydrate (by hydrolysis) 38.4%	Pyridoxine — 3.14 (rat growth)
	Pantothenic acid — 5.2 (bacterial growth)
	Inositol — 644.0 (yeast growth)
	Niacin — 30.0 (bacterial growth)

The dogs were fed the diet plus any vitamin supplements six times per week and always on the day preceding an experiment. Any dog suspected of coprophagy was muzzled in such a way as to prevent this, yet to permit the dog to drink water, which was provided ad libitum. The general program of feeding was to produce the deficiency, add the pantothenate supplement, reproduce the deficiency by removing the supplement, repeat the test of pantothenate supplement, and then testing the effects of other factors, including pyridoxine and inositol. The periods of deficiency and supplementation were alternated several times to permit detection of progressive changes.

In all periods the diets were supplemented with adequate amounts of the oil soluble vitamins A, D, E, and K, as follows: A, 200 I.U./kg./day; D, 10 I.U./kg./day; E, 1 mg./kg./day; K, 1 mg./kg./day.

The test meal consisted of 100 gm. of thoroughly dried and ground experimental diet, and was fed with 250 cc. of water at the beginning of each experiment, after the pre-feeding collection had been made. If a dog on any day refused to eat the entire test meal or if, for any reason, such as undue excitement or restlessness, the dog was considered not to be in a normal state, the experiment was discarded.

Analyses were made both for reducing sugar and total carbohydrate by the Shaffer-Somogyi ('33) method, and for diffusible and total nitrogen by the Kjeldahl method. From these data and from analysis of the test meal the amounts of carbohydrate and protein which had been digested and absorbed were calculated.

RESULTS

1. Motility

It was found that the gastrointestinal motility decreased in two dogs maintained on the whole wheat bread diet supplemented only with the oil-soluble vitamins, and that additional pantothenic acid was necessary for the proper maintenance of that motility.

Dog 1 showed an average emptying time of 5.9 hours for the basal period of 4 weeks on the complete basal diet (fig. 1). When the animal was fed the experimental diet, the emptying time gradually increased in 7 weeks to an average of 11.8 hours. The administration of calcium pantothenate [3] at a level of 220 μg. per kilogram of body weight per day was begun after 9 weeks on the experimental diet. During the week following the beginning of administration of the vitamin, the emptying time decreased from an average of 11.8 hours (three experiments) to a normal average of 5.0 hours (two experiments). Using Fisher's ('38) *t* test the probability of this difference occurring by chance was calculated to be less than 1 in 100. Unfortunately, this animal later required a repair

[3] Kindly supplied by Merck and Company.

operation due to a closing fistula and died post-operatively. Death was not ascribed to the deficiency.

Dog 2, having been on the experimental diet nearly a month before experiments were begun, was already slightly deficient. The emptying time for the next 3 weeks increased to an

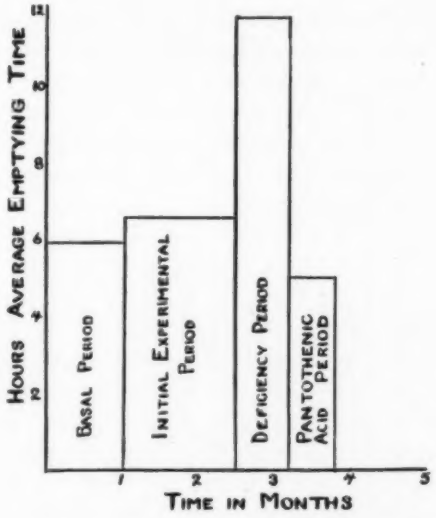

Fig. 1 Average emptying times for dog 1.

average of 10.4 hours (fig. 2). Within a week of calcium pantothenate administration at the same level as for dog 1, the emptying time dropped to a normal average of 5.6 hours over a period of a month. Upon removal of the pantothenate supplement the emptying time again gradually increased, reaching an average of 12.2 hours in 2½ months. Administration of the pantothenic acid at two later periods when the dog was in the deficient state resulted in each case in a prompt return of the motility to normal. On the basis of these combined periods the average emptying time during the deficiency periods was 11.5 hours (fourteen experiments) as compared to 6.4 hours for the vitamin control periods (nine experi-

ments). Again the calculated probability of chance was less than 1 in 100. Hence, a deficiency of pantothenic acid in one period with dog 1 and in three different periods with dog 2 resulted in an average increase in emptying time above normal of 100% and 80%, respectively (figs. 1 and 2). Addition of 220 μg. of calcium pantothenate per kilogram per day restored the motility to normal. It appears likely, therefore, that the adult dog's daily pantothenic acid requirement lies somewhere

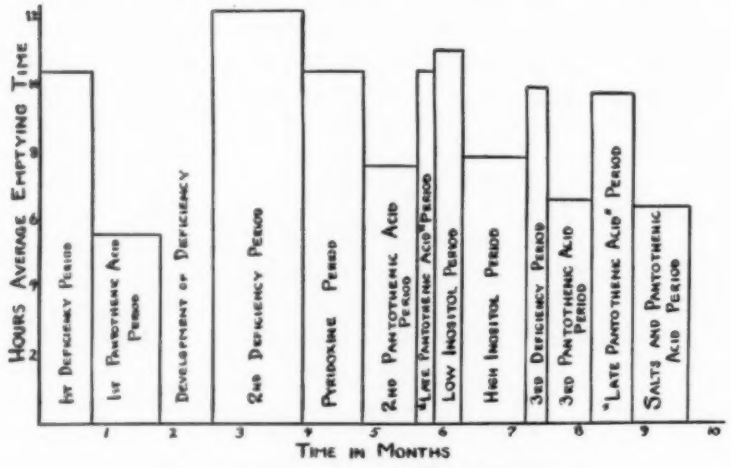

Fig. 2 Average emptying times for dog 2.

between 130 μg./kg. of body weight, the amount supplied by the bread, and 350 μg./kg. of body weight.

During pyridoxine administration (40 μg./kg./day) for a period of 1 month no significant effect on the deficient G. I. functions could be demonstrated, the average emptying time for the period being 10.4 hours for four completed runs, or 60% over the basal level. In three other attempted runs, the dog vomited, having already retained the test meal in the stomach (very small collections, if any, through the catheter) for 7–10 hours after feeding. During this period healings of a lesion on one leg and of a lesion in the mouth were noted,

though these lesions had not been ascribed to a pyridoxine deficiency.

Martin et al. ('41) mentioned constipation in inositol-deficient mice, and reported that in dogs on a constipating though nutritionally adequate diet, inositol, as studied roentgenologically, had a marked stimulatory effect on intestinal peristalsis. Anderson ('16) had noted that inositol dosing caused a transitory diarrhea in man and dogs. Woolley ('42) has reported the intestinal synthesis of inositol in mice. He also reported that in vitro synthesis by intestinal flora did not proceed in the absence of pantothenic acid, and again ('41) that when sufficiently high levels of pantothenic acid were fed, alopecia frequently did not appear in inositol-deficient mice. Hence, it was thought that inositol might be tried to determine whether or not pantothenic acid acted indirectly by stimulating inositol production.

Subcutaneous administration of inositol (up to 33 mg./day) had no effect on the reduced intestinal motility (fig. 2), as would be expected with the large (though partially unavailable as phytin) content of inositol already present in the bread diet (table 1). Subsequent oral administration of up to 500 mg. per day of inositol resulted in reduction in the emptying time, one experiment being only 4½ hours long. However, as Anderson had noted with a normal diet, the effect was transitory and the length of the time again increased to over 10 hours in 2 weeks.

After 5 to 6 months on the bread diet, dog 2 began to show the effects of a secondary deficiency. After 3 weeks or so of the pantothenic acid supplementation, the emptying time tended to increase, despite continued administration of the vitamin. Increasing the dosage of the vitamin had no effect on this tendency of the "late pantothenic acid" runs. A complicating salt deficiency seemed probable; hence, 8 gm. per day of the basal salt mixture was added to the diet of dog 2 which was already receiving 3.30 mg. of pantothenic acid per day. Within a few days the emptying time decreased to an average of 6.2 hours (five experiments) — a value nearly

identical with the early value for pantothenic acid alone (fig. 2). It was concluded that there had been a complicating salt deficiency which had grown more and more severe over the months the animal was kept on the experimental diet.

Other than the effect on motility, there were interesting and possibly significant results. During the first deficiency period for each dog, as the emptying time increased, the normal sweet smell of chyme was replaced by a foul odor identified as that of hydrogen sulfide. This was not due to back seepage of gas from the colon of the atonic gut because, upon standing, the concentration of the gas in a given collection markedly increased. The odor of the H_2S disappeared during the periods of pantothenic acid feeding, but reappeared in dog 2 when the vitamin supplement was omitted from the diet. The muzzle worn by the dog eliminated the possibility of coprophagy. From twelve deficiency experiments, an average of 0.4 mg. of H_2S per experiment was obtained by the use of an aeration train. Pyridoxine was ineffective in reducing the amount of H_2S produced.

Other observations tended to fit in with a general gastrointestinal atony. During the deficient periods, the "buttons" of the fistulae became notably flabby and atonic, and leaks from the fistulae developed.

2. Digestion and absorption of carbohydrate and protein (see table 2)

It was found, in general, that the average total amounts of carbohydrate and protein digestion and absorption stayed remarkably constant during the different periods, the exceptions being the periods of the high inositol supplementation and of the salts and pantothenic acid supplementation. Marked differences in the rates were found, however, calculations being based for dog 1 on the eight basal runs, and for dog 2 on the nine runs of early pantothenic acid supplementation.

In both dogs, the average rates of carbohydrate and protein digestion and absorption show marked and statistically significant decreases during pantothenic acid deficiency. In dog 1

the digestion rates dropped to only one-half of the normal, while the absorption rates dropped to less than half. The probability of this difference being due purely to chance is less than 1 in 100. The data for dog 2 show similar differences, though not quite as great, for the rates of digestion and absorption between the average of the three different periods of pantothenic acid deficiency and that for the three corresponding periods of pantothenic acid supplementation (figs. 1 and 2, table 2). The differences in rate between basal and pantothenic acid periods in dog 1 are not significant. Pyridoxine supplementation did not alter the course of the deficiency.

Inositol exhibited a marked influence on the rates of carbohydrate and protein digestion and absorption. Relatively small doses (up to 33 mg./day) had no significant effects. Large oral doses (up to 500 mg./day or 32 mg./kg./day), however, exerted marked effects. The rate of carbohydrate digestion dropped to 58%, and that of protein digestion to 44% of the average rate in the pantothenic acid period; in each case this was lower than the rates of the deficiency periods. The average rates of absorption dropped much more, that of carbohydrate to 37% and that of protein to only 17% of the rate in the period on pantothenic acid supplement, both obviously highly significant decreases.

The irritating effect of inositol on the G. I. tract seems to be well-illustrated here, although an attempt was made to minimize the cathartic action by giving the inositol only with the regular daily meal and not with the test meal. A study of the protein absorption reveals this effect most clearly. The very low average total absorption and rate of absorption, coupled with the fact that two of the four analyzed runs showed rates of —.3%/hour and —1.2%/hour absorption, respectively, indicate marked stimulation of secretion, and perhaps diminished capacity to absorb water, resulting in the recovery of more nitrogen than had been ingested. This seems confirmed by the significantly larger total collection volumes

TABLE 2

Results on digestion and absorption.

DOG NO. AND PERIOD	NO. OF RUNS	LENGTHS (HRS.) OF EMPTYING TIMES	CARBOHYDRATE								PROTEIN							
			Digestion				Absorption				Digestion				Absorption			
			Total (gms.)	%/hour	% from basal	p (Fisher)	Total (gms.)	%/hour	% from basal	p (Fisher)	Total (gms. N)	%/hour	% from basal	p (Fisher)	Total (gms. N)	%/hour	% from basal	p (Fisher)
Number 1																		
Basal [1]	8	5.9	30.8	10.1	....	...	35.5	10.4	....	...	2.16	14.3	....	...	1.04	6.9	....	...
Deficiency	3	11.8	34.0	5.2	— 48	.01	33.4	4.4	— 58	.01	2.20	7.1	— 50	.01	.98	3.1	— 55	.01
Pantothenic acid supplement	2	5.0	33.7	13.0	+ 29	.2	29.5	9.9	— 5	.8	2.28	17.6	+ 23	.3	.94	7.2	+ 4	.8
Number 2																		
Pantothenic acid supplement [2]	9	6.4	36.4	9.8	....	...	34.7	8.4	....	...	1.96	12.3	....	...	1.08	6.7	....	...
Deficiency	14	11.5	37.9	6.2	— 37	.01	37.6	5.4	— 36	.01	1.90	6.5	— 47	.01	1.00	3.4	— 49	.01
Pyridoxine supplement	4	10.4	36.4	6.5	— 34	.02	38.2	6.3	— 25	.05	1.81	6.8	— 45	.01	.99	3.5	— 48	.05
Inositol (small am'ts.) suppl.	4	11.0	40.0	7.1	— 28	.05	35.7	6.3	— 25	.05	1.65	6.4	— 48	.01	.96	3.7	— 45	.07
Inositol (large am'ts.) suppl.	4	7.9	26.4	5.7	— 42	.01	16.8	3.1	— 63	.01	1.06	5.4	— 56	.01	.24	1.1	— 83	.01
Number 2 "Late pantothenic acid" runs	6	9.7	35.2	6.2	— 37	.01	31.4	5.1	— 39	.01	1.61	6.4	— 48	.01	.96	3.4	— 49	.02
Salts supplement	5	6.1	31.9	8.8	— 10	.1	23.2	5.9	— 30	.05	1.27	8.1	— 34	.01	.38	2.4	— 64	.01

[1] Exclusive of this period, experimental diet was used in the daily feeding. Experimental test meals were used in all the runs.

[2] 220 μg./kg./day.

(table 3). Wasteneys and co-workers ('41) have briefly discussed the difficulty of studying the true or absolute digestion and absorption of an ingested protein. It is of interest to note (table 3) that the ratio of total recovered nitrogen to the total collection volume remains relatively constant, even with inositol, showing a practically linear relationship throughout. Since digestive secretions contain little or no reducing substance, no complication from this cause enters into the interpretation of the data for carbohydrate.

TABLE 3

Average total collection volume and recovered nitrogen.

EXPERIMENT		TOTAL NITROGEN ABSORPTION		TOTAL COLLECTION VOLUME	NITROGEN RECOVERED IN COLLECTION
Type	Number				
		gm.	*%/hr.*	*cc.*	*mg./cc.*
Pantothenic acid supplement	9	1.08	6 7	450	3.6
Deficiency	14	1.00	3.4	500	3.3
Inositol	4	.24	1.1	705	3.2

Paradoxically enough, the dog lost no weight during the month of inositol supplementation and the accompanying poor absorption but rather showed a slight gain, the average weight during this period being 16.3 kg., and during the other periods, 15.7 kg. This suggests higher, compensatory absorption of the daily diet in the lower jejunum and ileum.

The whole response to inositol does not appear to be that of the relief of a deficiency of that substance, but rather, of a normal effect superimposed upon an unrelated deficiency.

In the "late pantothenic acid" runs discussed with regard to motility, the rates of digestion and absorption dropped back towards deficiency levels again, showing the existence of a long progressive secondary deficiency. Addition of salts did not relieve this deficiency, although the motility returned promptly to normal. Though the rates of carbohydrate digestion and absorption and protein digestion showed slight improvement, the total amounts of carbohydrate and protein digested and absorbed were lower, especially in the case of protein absorption.

CONCLUSIONS

In enterostomized dogs maintained exclusively on a peeled whole wheat bread diet, a severe deficiency was produced in the course of 2 to 3 months. This was characterized by an approximately 50% decrease in gastrointestinal motility, accompanied by 40–60% decreases in the rates of carbohydrate and protein digestion and absorption. The almost immediate effect of adding a supplement of 220 μg. of calcium pantothenate per kilogram of dog weight per day to this diet was the return of these functions to normal in every case. The total digestion and absorption remained practically the same in the normal deficient states.

Pyridoxine was ineffective in altering the course of the deficiency.

Inositol acted more as a cathartic, its effect being apparently superimposed on the deficiency. While the motility was temporarily improved, the total amounts and rates of digestion and absorption were greatly decreased. A suggestion of synergism between inositol and pantothenic acid was not confirmed by this method.

After 5 to 6 months on the experimental diet, a secondary deficiency was noticed, characterized by diminished effectiveness of continued pantothenic acid supplementation to maintain the normal functions of motility, digestion and absorption. Adding a salt supplement, together with the continued pantothenic acid supplement, resulted in a prompt return of the motility to normal but only in very slight improvements in digestion and absorption rates.

LITERATURE CITED

ANDERSON, R. J. 1916 The utilization of inosite in the dog. J. Biol. Chem., vol. 25, p. 391.

ARNOLD, A., AND C. A. ELVEHJEM 1939 Influence of the composition of the diet on the thiamin requirement of dogs. Am. J. Physiol., vol. 126, p. 289.

FISHER, R. A. 1938 Statistical methods for research workers. Oliver and Boyd, London.

FROST, D. V., C. A. ELVEHJEM AND E. B. HART 1941 A study of the need for cobalt in dogs on milk diets. J. Nutrition, vol. 21, p. 93.

LAMBOOY, J. P., AND E. S. NASSET 1943 The inadequacy of eight synthetic B vitamins for the nutrition of puppies — unknown factor (factors) in yeast and probably liver. J. Nutrition, vol. 26 (in press).

MCKIBBIN, J. M., S. BLACK AND C. A. ELVEHJEM 1940 The essential nature of pantothenic acid and another alkali labile factor in the nutrition of the dog. Am. J. Physiol., vol. 130, p. 365.

MARTIN, G. J., M. R. THOMPSON AND J. D. CARVAJAL-FORERO 1941 Influence of inositol and other B-complex factors upon the motility of the G. I. tract. Am. J. Dig. Dis., vol. 8, p. 291.

RUSSELL, R. S., AND E. S. NASSET 1941 The effects of various vitamin supplements and of whole yeast on the digestion and absorption of the carbohydrate of a complete diet. J. Nutrition, vol. 22, p. 287.

SCHAEFER, A. E., J. M. MCKIBBIN AND C. A. ELVEHJEM 1942 Pantothenic acid deficiency studies in dogs. J. Biol. Chem., vol. 143, p. 321.

SEALOCK, R. R., AND A. LIVERMORE 1943 The vitamin B-complex of peeled wheat bread. J. Nutrition, vol. 25, p. 265.

SHAFFER, P. A., AND M. SOMOGYI 1933 Copper-iodometric reagents for sugar determinations. J. Biol. Chem., vol. 100, p. 695.

WASTENEYS, H., B. F. CROCKER AND R. HAMILTON 1941 Studies of digestion in the dog. Am. J. Physiol., vol. 135, p. 6.

WOOLLEY, D. W. 1941 Identification of the mouse anti-alopecia factor. J. Biol. Chem., vol. 139, p. 29.

———— 1942 Bacterial synthesis of inositol and its importance in the nutritional economy in mice. J. Bact., vol. 43, p. 5.

FURTHER CONSIDERATION OF THE EFFECT OF ALTITUDE ON BASAL METABOLISM

A STUDY ON YOUNG WOMEN RESIDENTS OF DENVER [1]

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ONE FIGURE

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The determination of the effect of altitude on the basal metabolism of human subjects has been the object of numerous investigations. The usual procedure has been to determine the basal metabolism on the same subjects at one or more different altitudes. However, in a few instances this problem has been studied by comparing the results obtained on residents of one altitude with those of similar investigations at other altitudes. Thus, McKittrick ('36) has obtained higher values for the basal metabolism of young women residents of Laramie, Wyoming (altitude 7,148 feet) than those reported in certain other similar investigations at lower altitudes. Moreover, McCrery and Wolf ('38) and McCrery, Wolf and Bavousett ('40) have claimed that the basal metabolism of young women of Lubbock, Texas (altitude 3,200 feet) lies between the values found by McKittrick and those reported for elevations below 1,000 feet. The workers at both altitudes have concluded that their respective data indicate an influence of altitude on basal metabolism. On the other hand, Lewis, Iliff, Duval and Kinsman ('43) have found that the basal

[1] This report is taken from the dissertation submitted by Alberta Iliff to the Faculty of the Graduate School of the University of Colorado, June, 1942, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

metabolism of the same subjects determined at widely different elevations (910 feet, 5,280 feet and 8,720 feet) by the same workers with the same portable closed circuit apparatus (a no. M 84 McKesson Metabolor) does not change significantly as a result of altitude. On account of the divergence between these results and those reported by McKittrick and by McCrery and co-workers, further consideration of this problem seems desirable. Accordingly, unpublished data on the basal metabolism of young women residents of Denver are herewith presented and compared with those of like observations obtained at different altitudes by other investigators.

EXPERIMENTAL PROCEDURE

During the past several years, ninety determinations of basal metabolism have been made on forty-three healthy women between the ages of 17 and 26 years, inclusive, who had been residing in Denver (altitude 5,280 feet) for at least 1 year before the observations were made. The subjects had fasted for a period of from 12 to 15 hours and had rested quietly for 45 minutes immediately prior to the determinations, which were made either with a closed circuit bedside type of apparatus, a no. M 84 McKesson Metabolor, or by the open circuit chamber procedure of Higgins and Bates ('30). The latter method has been used in the metabolism laboratory of the Child Research Council for the past 11 years and has been described in detail by Lewis, Kinsman and Iliff ('37). The basal metabolism of each subject was determined on at least 2 different days. In the seven instances in which the same individual was studied at different ages, the determinations are reported separately for each age just as if they had been obtained on different subjects.

Alcohol check determinations were made at intervals during the study on both the open circuit and the closed circuit apparatus. The values obtained for oxygen consumption varied from the theoretical by $\pm 2\%$ with the chamber procedure, and by $\pm 3\%$ with the closed circuit method.

TABLE 1

Individual data on basal metabolism.

SUBJECT	NUMBER OF TESTS	AGE	WEIGHT	HEIGHT	SURFACE AREA	CAL./ 24 HRS.	CAL./HR./ SQ. M.	CAL./HR./ KG.	CAL./HR./ CM.
		years	*kg.*	*cm.*	*q. m.*				
1	1	17	65.0	160.9	1.680	1313	32.6	0.842	0.340
2[1]	1	17	60.0	173.3	1.720	1375	33.3	0.955	0.330
3[1]	1	17	54.4	173.6	1.650	1391	35.1	1.07	0.334
4[1]	1	17	65.0	168.8	1.745	1432	34.2	0.918	0.353
5	2	18	54.6	168.4	1.620	1311	33.8	1.00	0.324
6	2	18	50.8	156.8	1.490	1297	36.3	1.06	0.344
1	1	18	63.6	161.7	1.670	1299	32.4	0.851	0.335
7	2	18	48.5	167.8	1.530	1204	32.8	1.04	0.299
8	2	19	65.4	170.9	1.765	1361	32.2	0.867	0.332
9	2	19	50.8	160.8	1.515	1230	33.8	1.01	0.319
10	2	19	37.2	150.4	1.265	1098	36.2	1.23	0.304
11	2	19	54.2	172.6	1.640	1276	32.4	0.980	0.308
12	2	20	50.4	176.7	1.620	1330	34.3	1.10	0.314
13	2	20	58.4	167.8	1.660	1322	33.2	0.944	0.328
14	2	20	52.0	175.7	1.630	1282	32.7	1.02	0.304
15	2	20	51.6	165.1	1.560	1321	35.4	1.06	0.334
16	3	21	53.1	168.6	1.600	1130	29.4	0.886	0.279
17	2	21	38.4	149.6	1.275	1066	34.9	1.16	0.279
18	2	21	39.0	148.3	1.275	1087	35.6	1.16	0.306
19	2	21	62.4	172.9	1.740	1364	32.6	0.912	0.329
20	2	22	51.6	162.8	1.540	1138	30.8	0.918	0.292
21	1	22	70.3	186.9	1.940	1443	31.0	0.855	0.322
22	2	22	61.8	175.2	1.755	1136	27.0	0.766	0.270
23	2	22	50.2	167.2	1.550	1173	31.5	0.974	0.292
24	2	23	61.6	175.6	1.750	1234	29.3	0.834	0.292
25	2	23	57.4	160.5	1.595	1187	31.0	0.862	0.308
26	2	23	48.2	162.0	1.490	1190	33.3	1.03	0.306
27	2	23	64.8	167.8	1.735	1366	32.8	0.878	0.340
28	1	23	61.0	170.8	1.715	1256	30.5	0.858	0.306
28	1	24	58.7	170.4	1.680	1186	29.4	0.842	0.290
21	3	24	71.5	186.9	1.950	1419	30.3	0.827	0.317
29	2	24	48.6	166.2	1.520	1097	30.0	0.940	0.275
30	3	24	52.7	169.8	1.605	1169	30.4	0.924	0.287
31	1	24	50.3	158.4	1.490	1048	29.3	0.868	0.276
32	2	24	51.2	163.2	1.535	1160	31.4	0.944	0.296
33	2	24	51.7	167.4	1.570	1172	31.0	0.944	0.292
34	2	24	55.3	177.1	1.685	1266	31.4	0.954	0.298
35	3	24	45.3	165.8	1.475	1169	33.1	1.08	0.293
35	1	25	44.2	165.8	1.455	1085	31.1	1.02	0.273
29	1	25	48.0	166.8	1.520	1073	29.4	0.932	0.268
31	1	25	51.9	158.2	1.510	1043	28.8	0.838	0.275
36	1	25	54.2	166.7	1.600	1167	30.4	0.897	0.292
37	2	25	67.2	161.7	1.710	1256	30.6	0.780	0.324
38[1]	1	26	45.8	159.0	1.435	1111	32.3	1.01	0.291
36	1	26	54.3	166.9	1.605	1146	29.8	0.879	0.286
39	3	26	59.9	173.7	1.720	1326	32.1	0.922	0.318
40	2	26	54.8	167.9	1.615	1116	28.8	0.849	0.277
41	2	26	59.2	172.6	1.700	1218	29.8	0.858	0.294
42	2	26	54.2	165.4	1.590	1152	32.8	0.962	0.316
43	2	26	57.8	170.9	1.670	1193	29.8	0.860	0.291

[1] The other determinations on these four subjects are not given in this table since they were made at ages outside the range reported in this study.

RESULTS

The basal metabolism expressed as calories per 24 hours, calories per hour per square meter, calories per hour per kilogram and calories per hour per centimeter for each subject is given in table 1. This table also gives the age, weight, height and surface area of each subject. The results are listed in the order of the age attained at the last birthday. When two or more determinations were obtained on the same individual at the same age, the mean values for this age are reported. The surface area is taken from the nomogram of Wilson and Wilson ('20) which is based on the height-weight formula of Du Bois and Du Bois ('16). Since it has been found (Lewis, Iliff and Duval, '43) that the closed circuit apparatus gives results which agree closely with those obtained with the chamber procedure, no designation has been made as to which type of apparatus was used with each subject.

The means, standard deviations and coefficients of variation for calories per hour per square meter, calories per hour per kilogram and calories per hour per centimeter, respectively, have been calculated for the young women residents of Denver for each half of the age range studied, namely, from 17 to 21 years, inclusive, and from 22 to 26 years, inclusive, and for the entire range of from 17 to 26 years, inclusive. These values are reported in table 2. As would be expected, since these three

TABLE 2

The mean, standard deviation and coefficient of variation for calories per hour per square meter, calories per hour per kilogram and calories per hour per centimeter obtained on the young women residents of Denver for the age ranges indicated.

	17 TO 21 YEARS, INCLUSIVE			22 TO 26 YEARS, INCLUSIVE			17 TO 26 YEARS, INCLUSIVE		
	Cal./hr./sq. m.	Cal./hr./kg.	Cal./hr./cm.	Cal./hr./sq. m.	Cal./hr./kg.	Cal./hr./cm.	Cal./hr./sq. m.	Cal./hr./kg.	Cal./hr./cm.
Mean	33.7	1.003	0.320	30.6	0.904	0.295	31.8	0.943	0.305
Standard deviation	1.6	0.107	0.019	1.4	0.072	0.018	2.1	0.100	0.022
Coefficient of variation	4.8	10.6	5.9	4.6	8.0	6.1	6.5	10.6	7.4

values decrease with increasing age, the means for calories per hour per square meter, calories per hour per kilogram and calories per hour per centimeter are lower in the 22 to 26 year age group than in the 17 to 21 year period. The decrease in these values with increasing age will also explain why, except in two instances, the standard deviations and the coefficients of variation are lower for the 5 year period than for the 10 year period. When the values are considered from the standpoint of which method is the best for comparing the basal metabolism, it is evident that the coefficients of variation show a definitely lower dispersion for calories per hour per square meter than for calories per hour per kilogram and for calories per hour per centimeter. Accordingly, the first value, calories per hour per square meter, will be the only method used in the comparison with the literature that is to follow.

DISCUSSION

If altitude is a factor which raises the basal metabolism, as contended by McKittrick ('36) and by McCrery, Wolf and Bavousett ('40), the values for basal metabolism found in the present study should fall approximately midway between those obtained at Laramie and at Lubbock, which are about 2,000 feet higher and lower, respectively, than the elevation of Denver. However, that this is not the case may be seen from figure 1, which gives the mean values for the basal metabolism of the young women residents of Denver and those obtained elsewhere during the last 15 years in similar investigations on women between 17 and 26 years of age, inclusive. The mean of 31.8 calories per hour per square meter obtained at Denver is even lower than the 33.5 calories found by McCrery, Wolf and Bavousett instead of being approximately halfway between this latter value and that of 35.6 calories found by McKittrick. Thus, the data herein presented do not support the thesis that altitude causes an increase in basal metabolism.

Obviously these divergent results concerning the effect of altitude on basal metabolism need clarification. The present

study supports the results of Lewis, Iliff, Duval and Kinsman ('43), who found no effect of change of altitude on the basal metabolism. Since both of these studies are in agreement with the literature on the subject, the question is raised as to whether McKittrick ('36), McCrery and Wolf ('38) and

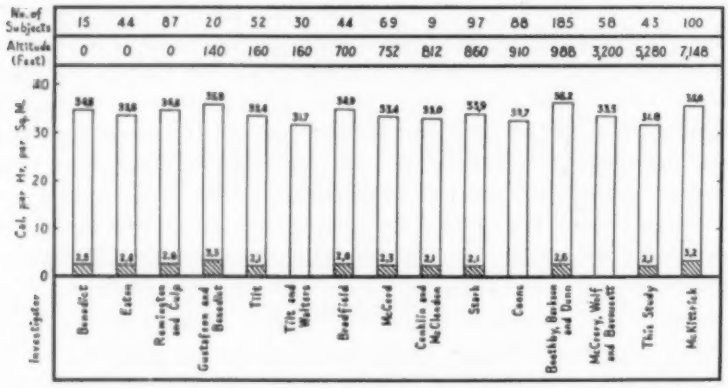

Fig. 1 Graphic comparison of the basal metabolism found in the present study and by other investigators for young women between 17 and 26 years of age, inclusive. The long rectangular column for each study shows the mean basal metabolism expressed as calories per hour per square meter. Where possible from the data given,[2] the standard deviation has been calculated for each study and is shown by the shaded area at the bottom of each column. The exact values for each mean and its standard deviation are given at the top of the respective columns which depict them. At the top of the graph above each column are to be found the number of subjects studied and the altitude at which each study was made.

[2] We are indebted to Dr. Juliana S. McCord for furnishing detailed records so that we could separate her data in the age range between 17 and 27 years of age from those of older women.

McCrery, Wolf and Bavousett ('40) are justified in drawing the conclusion that altitude affects the basal metabolism.

McKittrick ('36) compared the results of the determinations of basal metabolism which she obtained on young women residents of Laramie (altitude 7,148 feet) with those found by Tilt ('30) at Tallahassee, Florida (altitude 160 feet) and by Coons ('31) at Stillwater, Oklahoma (altitude 910 feet).

As may be seen from figure 1, the value reported by McKittrick is definitely higher than that obtained either by Tilt or by Coons. For this reason McKittrick concluded that the high value found in Laramie indicated that the high altitude at which these determinations were made raised the basal metabolism. Besides the literature to which McKittrick made reference, reports of several other investigators were then available for comparison. Tilt and Walters ('35) at Tallahassee (altitude 160 feet), Conklin and McClendon ('30) at Minneapolis (altitude 812 feet) and Stark ('32, '33) at Madison, Wisconsin (altitude 860 feet) agreed with Tilt and with Coons in that their mean values were definitely lower than that found by McKittrick. However, Gustafson and Benedict ('28) at Wellesley, Massachusetts (altitude 140 feet) and Boothby, Berkson and Dunn ('36) at Rochester, Minnesota (altitude 988 feet) obtained values very similar to that found by McKittrick. Furthermore, Benedict ('28) at Boston, Massachusetts (sea level), Remington and Culp ('31) at Charleston, South Carolina (sea level) and Bradfield ('27) at Columbia, Missouri (altitude 700 feet) reported that residents of these respective localities showed mean values for calories per hour per square meter which were only 1 calorie or less below that found by McKittrick. The lack of correlation between the basal metabolism found and the altitude at which the studies were made does not justify the conclusion that the relatively high basal heat production obtained on young women at Laramie is attributable to altitude.

McCrery and Wolf ('38) found the basal metabolism of young women at Lubbock (altitude 3,200 feet) when expressed as calories per hour per square meter to be lower than that reported by McKittrick ('36) at Laramie (altitude 7,148 feet) and higher than that obtained by Tilt ('30) at Tallahassee (altitude 160 feet). This work was expanded by McCrery, Wolf and Bavousett ('40), who compared the total calories found on young women residents of Lubbock with those obtained by Tilt, by Coons ('31) at Stillwater (altitude 910 feet), by Stark ('32, '33) at Madison (altitude 860 feet) and by

McKittrick. Since the values procured at Lubbock fell between those reported by Tilt, by Coons and by Stark, on the one hand, and those found by McKittrick, on the other, McCrery, Wolf and Bavousett concluded that their results indicated that altitude raises the basal metabolism. However, their procedure of comparing total calories does not take into account the size of the individual. When calories per hour per square meter are considered, the results of McCrery and co-workers do not show significant differences from those obtained by Tilt, by Coons and by Stark. Moreover, the mean value of 33.5 calories per hour per square meter obtained on young women of Lubbock is lower than those found by Benedict ('28) at Boston (sea level), by Remington and Culp ('31) at Charleston (sea level), by Gustafson and Benedict ('28) at Wellesley (altitude 140 feet), by Bradfield ('27) at Columbia (altitude 700 feet) and by Boothby, Berkson and Dunn ('36) at Rochester (altitude 988 feet); it is practically the same as those found by Eaton ('39) at New Orleans (sea level) and by McCord ('39) at Bloomington, Indiana (altitude 752 feet), and only 0.5 of a calorie higher than that found by Conklin and McClendon ('30) at Minneapolis (altitude 912 feet). Consequently, the conclusion of McCrery, Wolf and Bavousett that their results indicate that altitude raises the basal metabolism is unwarranted.

Consideration of the standard deviations for the basal metabolism reported by the different workers may throw some light on whether variation in the degree of dispersion of the data is a factor in the discrepancies observed. It will be seen from figure 1 that the values for standard deviation vary between 2.1 and 3.3 calories per hour per square meter and are within 0.5 of a calorie of one another, if the results of Gustafson and Benedict ('28) and McKittrick ('36) are excepted. The relatively high degree of dispersion of the data in these two studies may well explain why these investigators found higher values for calories per hour per square meter than any of the others cited with the exception of those of Boothby, Berkson and Dunn ('36). Except for the two studies

noted, the degree of dispersion is very similar; hence, a comparison of the means should give an accurate picture of whether the basal metabolism is affected by altitude. A glance at figure 1 shows that no consistent relationship exists between the basal metabolism of the young women observed and the altitude at which the studies were made. This is even more evident, if one considers specific results. For example, the mean of 31.8 calories per hour per square meter obtained in the present study at Denver (altitude 5,280 feet) is similar to the 31.7 calories found by Tilt and Walters ('35) at Tallahassee (altitude 160 feet) and is lower than the values reported by the other investigators whose studies were made at elevations below 1,000 feet. Moreover, the value of 33.9 calories found by Stark ('32, '33) at an altitude of 860 feet is little different from the values obtained at a similar elevation by McCord ('39), at lower elevations by Eaton ('39) and by Tilt ('30) and at 3,200 feet by McCrery, Wolf and Bavousett ('40). Thus, a preponderance of evidence warrants the conclusion that, within the ranges of elevation cited herein, altitude does not affect the basal metabolism.

SUMMARY

1. Ninety determinations of basal metabolism on forty-three young women residents of Denver between the ages of 17 and 26 years, inclusive, are reported.

2. When the results of these determinations are compared with the values obtained in similar investigations at other altitudes during the last 15 years, the basal metabolism of the young women studied at Denver (altitude 5,280 feet) is found to agree closely with, or to be lower than, the observations on women of comparable ages at elevations below 1,000 feet.

3. No consistent relationship exists between the basal metabolism of the young women observed in all of these studies and the altitude at which the determinations were made.

4. The relatively high values for basal metabolism at the higher elevations that have been reported in the literature cited as due to altitude must be attributable to some other causative factor.

ADDENDUM

After this paper was accepted for publication, J. McCrery, M. Wolf Lamb and N. D. Bavousett published an article (J. Nutrition, vol. 25, p. 245, March, 1943) in which they state that the differences found in the basal metabolism of normal women of college age by various investigators "cannot be attributed to altitude." This conclusion is a complete reversal of the earlier opinion of these authors (McCrery, Wolf and Bavousett, '40) that the basal metabolism is affected by altitude.

LITERATURE CITED

BENEDICT, F. G. 1928 Basal metabolism data on normal men and women with some considerations on the use of prediction standards. Am. J. Physiol., vol. 85, p. 607.

BOOTHBY, W. M., J. BERKSON AND H. L. DUNN 1936 Studies of the energy of metabolism of normal individuals: A standard for basal metabolism, with a nomogram for clinical application. Am. J. Physiol., vol. 116, p. 468.

BRADFIELD, H. S. 1927 The determination of the surface area of women and its use in expressing basal metabolic rate. Univ. of Mo. Agric. Exp. Sta. Res. Bull. No. 109.

CONKLIN, C. J., AND J. F. MCCLENDON 1930 Basal metabolic rate in relation to menstrual cycle. Arch. Int. Med., vol. 45, p. 125.

COONS, C. M. 1931 The basal metabolism of Oklahoma women. Am. J. Physiol., vol. 98, p. 692.

DU BOIS, D., AND E. F. DU BOIS 1916 Clinical calorimetry. X. Formula to estimate the approximate surface area if height and weight be known. Arch. Int. Med., vol. 17, p. 863.

EATON, A. G. 1939 The basal metabolic rate of normal individuals in New Orleans. J. Lab. and Clin. Med., vol. 24, p. 1255.

GUSTAFSON, F. L., AND F. G. BENEDICT 1928 Seasonal variation in basal metabolism. Am. J. Physiol., vol. 86, p. 43.

HIGGINS, H. L., AND V. BATES 1930 New method for the determination of the basal metabolism of babies and small children. Am. J. Dis. Child., vol. 39, p. 71.

LEWIS, R. C., A. ILIFF AND A. M. DUVAL 1943 The comparative accuracy of the closed circuit bedside method and the open circuit chamber procedure for the determination of basal metabolism. J. Lab. and Clin. Med., vol. 28, p. 1238.

LEWIS, R. C., A. ILIFF, A. M. DUVAL AND G. M. KINSMAN 1943 The effect of change of altitude on the basal metabolism of human subjects. J. Lab. and Clin. Med., vol. 28, p. 851.

LEWIS, R. C., G. M. KINSMAN AND A. ILIFF 1937 The basal metabolism of normal boys and girls from two to twelve years old, inclusive. Am. J. Dis. Child., vol. 53, p. 348.

MCCORD, J. S. 1939 Basal metabolism of Indiana University women. J. Am. Diet. Assoc., vol. 15, p. 440.

MCCREERY, J., AND M. M. WOLF 1938 Basal metabolism of normal college women at an altitude of 3,200 feet. J. Home Econ., vol. 30, p. 583.

MCCREERY, J., M. M. WOLF AND N. D. BAVOUSETT 1940 Studies in basal metabolism of normal women. J. Home Econ., vol. 32, p. 562.

MCKITTRICK, E. J. 1936 Basal metabolism of Wyoming University women. J. Nutrition, vol. 11, p. 319.

REMINGTON, R. E., AND F. B. CULP 1931 Basal metabolic rate of medical students and nurses in training at Charleston, South Carolina. Arch. Int. Med., vol. 47, p. 366.

STARK, M. E. 1932 Standards for predicting basal metabolism. I. Standards for girls from 17 to 21. Trans. Wis. Acad. Sci., Arts and Letters, vol. 27, p. 251.

———— 1933 Standards for predicting basal metabolism. I. Prediction for girls from 17 to 21. J. Nutrition, vol. 6, p. 11.

TILT, J. 1930 The basal metabolism of young college women in Florida. J. Biol. Chem., vol. 86, p. 635.

TILT, J., AND C. F. WALTERS 1935 Basal metabolism and diet of normal young college women in Florida. J. Nutrition, vol. 9, p. 109.

WILSON, C. M., AND D. WILSON 1920 The determination of the basal metabolic rate and its value in diseases of the thyroid gland. Lancet ii, p. 1042.

A STUDY OF THE AVAILABILITY OF THE IRON IN ENRICHED BREAD

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TWO FIGURES

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The addition of iron compounds either to flour or directly to dough mixes as a part of the bread enrichment program has caused a renewed interest in the availability of different forms of iron to the organism. Forms of iron insoluble in neutral solution, such as the pyrophosphate, have been used to a considerable extent for flour or bread enrichment, because of their convenience in handling and the negligible effects of such compounds on the color, flavor, and texture of the bread. In view of the wide extent of the enrichment program the comparative availability of the different types of iron compounds used for this purpose becomes a factor of importance. This matter has been investigated by studying the availability to anemic rats of two widely different iron compounds which are of interest for bread enrichment purposes; one of these is soluble, and the other insoluble.

Sodium iron pyrophosphate was used as the insoluble type of iron, ferrous sulfate as the soluble form.

The effect of ferric pyrophosphate in milk anemia of rats has been previously investigated by Elvehjem, Hart and Sherman ('33), but the results obtained by these authors are not comparable to those noted in this study, since the Wisconsin workers used a salt of ferric pyrophosphate together with citric acid which rendered it soluble (Elvehjem, '42). (The Merck Index ('40) describes a "Ferric Pyrophosphate Soluble

N.F.VI." containing 12% Fe, 20% P_2O_5, and 40% citric acid, freely soluble in water.) This compound was found to be as available for hemoglobin regeneration as an equivalent amount of ferric chloride.

METHODS AND RESULTS

The anemic animals were prepared by rearing young rats on a ration of whole cow's milk, according to the procedure of Elvehjem and Kemmerer ('31). The animals were kept in rust-free galvanized iron cages, fitted with new wide mesh wire screens. No water was offered to the rats while on the milk ration, but those animals that were changed to a solid ration were supplied with water in glass bottles emptying into aluminum receptacles. The milk was fed in glass dishes.

In the first experiment, sodium iron pyrophosphate was compared with ferrous sulfate and ferric sulfate by administering the three compounds to groups of anemic rats which were maintained on a diet of whole milk throughout the test period. In the second experiment, after the production of milk anemia, the milk ration was discontinued and diets containing commercial iron-enriched bread were fed. The bread in one case contained sodium iron pyrophosphate, and in the other ferrous sulfate in an amount supplying exactly the same level of iron.

The sodium iron pyrophosphate [1] used was a double salt of the composition $Fe_4(P_2O_7)_3 \cdot 2\,Na_4P_2O_7 \cdot 6\,H_2O$. The actual iron content was found to be 15.99%, as compared to the theoretical value of 16.12%. The other salts used were Merck's Ferrous Sulfate Reagent ($FeSO_4 \cdot 7\,H_2O$) and Merck's Ferric Sulfate Reagent ($Fe_2(SO_4)_3$ with about $6\,H_2O$). The theoretical and observed iron content of the ferrous sulfate were 20.09% and 20.0%, for the ferric sulfate 21.99% and 23.8%, respectively.

Hemoglobin analyses were made by the acid hematin method, using a Klett-Summerson photoelectric colorimeter [2]

[1] Obtained from the Victor Chemical Works, Chicago, Ill.

[2] Made by the Klett Manufacturing Company, New York, N. Y.

for the measurement of the concentration of the solutions. The readings of this colorimeter were standardized with a series of acid hematin solutions made from a sample of blood of which the hemoglobin content was established by the oxygen capacity method of Lundsgaard and Möller ('22).

Iron analyses of the components of the diets were made by digestion in a mixture of nitric and perchloric acids, with development of color by α, α' dipyridyl as described in Cereal Chemistry Laboratory Methods ('41).

Activity of sodium iron pyrophosphate, ferrous sulfate, and ferric sulfate in anemic rats on a milk ration. The animals in this experiment were ready for testing at 7 weeks of age, with blood hemoglobin values of 2.8 to 4.5 (average 3.5) gm. per 100 cc. They were divided into groups of ten animals each and evenly distributed as to sex and average hemoglobin values. The pyrophosphate compound and the ferrous sulfate were given at two levels, supplying 0.15 or 0.30 mg. of iron per rat per day; the ferric sulfate was given only at the lower level. The ferrous and ferric sulfates were given in solution form; the sodium iron pyrophosphate was made into a 1% suspension with tragacanth. The solutions or suspension were administered into the animals' stomachs from a syringe by inserting a hypodermic needle tipped with a short fiber catheter. The preparations were given three times weekly in the amount of twice the "daily" dose. Some of the rats were rather feeble when placed on test, and a few died during the first week of the test period.

The average hemoglobin values for the various groups during the 4-week test period are shown in figure 1. It will be seen that the hemoglobin regeneration of 1.75 gm. in 4 weeks with 0.30 mg. of Fe as the pyrophosphate compound is considerably lower than the hemoglobin regeneration of 3.16 gm. noted in the series medicated with only 0.15 mg. of iron as ferrous sulfate.

These data indicate that sodium iron pyrophosphate is somewhat less than 50% as available for hemoglobin regeneration as ferrous sulfate under the experimental conditions used.

As would be expected, ferrous and ferric sulfates gave similar results. The ferric sulfate was tested in this experiment because of the still prevalent belief that clinically ferrous iron is much more effective than ferric iron salts. This finding of the similar availability of ferrous and ferric iron to anemic rats is in agreement with the report of Underwood ('38), and gives no support to the medical belief in the greater effectiveness of ferrous iron.

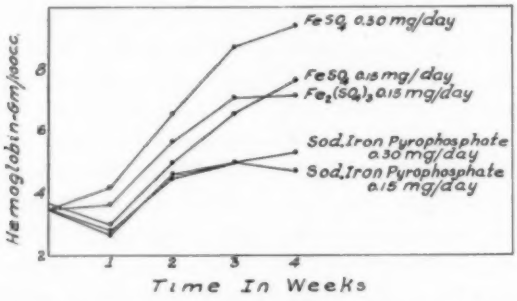

Fig. 1 Hemoglobin regeneration in anemic rats on a milk ration with different levels of ferrous sulfate, ferric sulfate, or sodium iron pyrophosphate. The figures at the right of each curve indicate the dosage levels in terms of milligrams of Fe per day. The curves are averages for the surviving seven to ten rats in each group.

Availability of the iron in enriched bread. Two lots of enriched bread, containing identical amounts of iron, were baked by a nationally known bakery. The iron contained in one lot of the finished product was in the form of sodium iron pyrophosphate, and in the other as ferrous sulfate.

Three diets were prepared from the dried and pulverized bread samples. All diets had the following percentage composition: dried bread 82, iron-low casein 12, cottonseed oil 3, and iron-free salt mixture 3. To each 100 gm. of the diet the following supplements were added: vitamin A concentrate 1500 I.U., viosterol 400 I.U., thiamine 400 μg., riboflavin 1200 μg., pyridoxine 400 μg., calcium pantothenate 4 mg., choline chloride 20 mg., inositol 20 mg., $CuSO_4 \cdot 5\,H_2O$ equivalent to 2 mg. Cu, and $MnCl_2 \cdot 4\,H_2O$, equivalent to 0.8 mg. Mn.

The iron-free salt mixture was prepared by modifying the U.S.P.XI ('36) salt mixture no. 2. The ferric citrate and sodium chloride were omitted and the sodium biphosphate replaced with potassium biphosphate, since bread contains adequate sodium chloride. The casein was included in the ration in order to insure a satisfactory protein composition, since the bread proteins are incomplete for satisfactory growth. It was found necessary to prepare this casein in the laboratory. Analysis of a sample of technical casein showed it to contain 0.045 to 0.190 mg. of iron per gram, irregularly distributed. A sample of commercial "vitamin-free" casein was found to contain similar amounts of iron. The laboratory-prepared casein had an iron content of 0.021 mg. per gram.

Diet 1 contained enriched bread prepared with sodium iron pyrophosphate; diet 2 contained bread prepared with ferrous sulfate; diet 3 contained an equal mixture of the above enriched breads with the addition of the iron supplement in the form of ferrous sulfate to the extent of 20 mg. of iron per 100 gm. of diet. Diet 3 was included for control purposes in order to prove that the basic ration would promote rapid hemoglobin production in the presence of optimum iron levels.

The iron contents of the enriched breads were found on analysis to be very similar, i.e., 0.0244 mg. per gram for the sodium iron pyrophosphate bread and 0.0249 mg. per gram for the ferrous sulfate bread. With the enriched bread constituting 82% of the ration and the casein (iron content 0.021 mg. per gram) 12% of the ration, it was calculated that 89% of the iron in the finished diet should be that furnished by the bread and 11% that furnished by the casein. Analysis of the finished diets indicated that the actual iron content agreed with the calculated content within 1%, showing freedom from contamination with iron from any other source.

Anemic rats were prepared as previously described and divided into three evenly-matched groups: fifteen rats each for diets 1 and 2; ten for diet 3. The average extent of hemoglobin regeneration on the different rations during the 4-week test period is indicated in figure 2. It will be seen that the

total 4-week gain in hemoglobin of 2.57 gm. per 100 cc. for diet 1 was somewhat less than half that of the 6.00 gm. gain for diet 2. This indicates that the iron in enriched bread containing iron as sodium iron pyrophosphate is less than 50% as available as that in enriched bread containing iron as ferrous sulfate. The very rapid increase of hemoglobin in group 3,

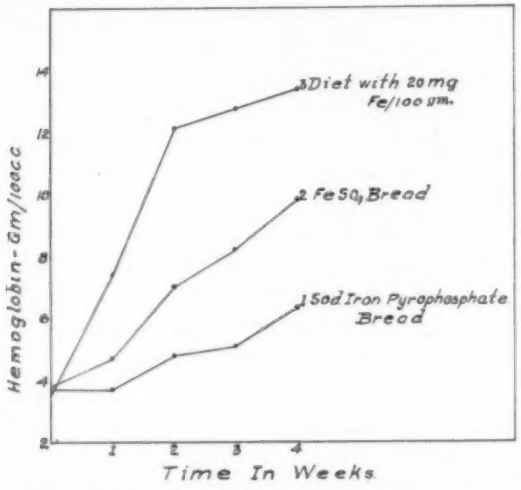

Fig. 2 Hemoglobin regeneration in anemic rats fed enriched bread. The curves are average figures for nine to fourteen rats per group.

fed a diet identical to that of groups 1 and 2, except that the iron content was ten times greater, indicates that the diet used will permit excellent hemoglobin regeneration in the presence of optimal iron.

It is, of course, necessary to know that the food consumption of the different groups of rats was similar, since the iron intake in this experiment was dependent upon the food intake. Food records kept during the first 3 weeks of the test period revealed that the average food consumption for groups 1, 2 and 3 was 11.0, 12.2 and 11.9 gm. per rat per day, respectively. The average growth rates for the three groups are shown in table 1.

TABLE 1

Growth rates of anemic rats fed enriched bread.

GROUP	NUMBER OF RATS COMPLETING TEST	INITIAL WEIGHT	AVERAGE WEIGHT AFTER			
			1 week	2 weeks	3 weeks	4 weeks
		gm.	*gm.*	*gm.*	*gm.*	*gm.*
1	14	66	87	108	128	137
2	11	65	94	115	137	145
3	9	64	93	115	138	146

It will be seen that both the food consumption and growth rates for groups 2 and 3 were practically identical, while the group 1 rats ate about 10% less food and grew a little more slowly. It is thus indicated that the much greater rate of hemoglobin regeneration in group 2 cannot be considered as due to the slightly greater food intakes.

Statistical evaluation of results. The data for the hemoglobin values at the end of the 4-week test period, both for the animals fed the enriched bread and those fed the iron salts, have been treated statistically in table 2.

TABLE 2

Availability of iron salts for hemoglobin production.

GROUP	MATERIAL FED	AMT. FE PER DAY	NO. OF RATS COMPLETING TEST	AVERAGE HEMOGLOBIN VALUES		
				Initial	Final (after 4-week test period)	Standard deviation of final hemoglobin values
		mg.		*gm./100 cc.*	*gm./100 cc.*	
	Experiment 1					
1	Sodium iron pyrophosphate	0.15	9	3.49	4.63	0.88
2	Sodium iron pyrophosphate	0.30	10	3.45	5.20	0.96
3	Ferrous sulfate	0.15	8	3.60	7.60	0.92
4	Ferrous sulfate	0.30	7	3.53	9.44	1.38
5	Ferric sulfate	0.15	8	3.51	7.06	1.82
	Experiment 2					
1	Sodium iron pyrophosphate bread		14	3.68	6.25	1.05
2	Ferrous sulfate bread		11	3.78	9.78	1.40
3	Diet with 20 mg. Fe per 100 gm.		9	3.53	13.36	1.24

In examining the results for significance, we may compare the final average hemoglobin value of group 1, fed sodium iron pyrophosphate in the amount of 0.15 mg. Fe per day, with that of group 3, fed ferrous sulfate also at the level of 0.15 mg. Fe per day. Dividing the difference of 2.97 in mean hemoglobin values by the standard deviation of 0.92, we get the value 3.23. Since the difference between groups is ordinarily considered significant when the mean difference is at least twice the magnitude of the standard deviation, the results may be considered indicative of a real significance. Comparing group 2, given 0.30 mg. Fe per day as sodium iron pyrophosphate with group 4, given 0.30 mg. Fe per day as ferrous sulfate, the mean difference in final hemoglobin values is 4.24. Dividing by 1.38, the standard deviation (S. D.) of the most variable group, we get 3.07 which may be taken as indicating a significant difference. There is also a significantly greater final hemoglobin value for group 3, receiving 0.15 mg. Fe as ferrous sulfate than for group 2, receiving 0.30 mg. Fe as sodium iron pyrophosphate, the difference in mean values being 2.50 S. D.

In experiment 2, the significance of the results is indicated by the fact that the mean final hemoglobin value for the group given bread baked from flour containing ferrous sulfate is 2.52 S. D. greater than that of the group given bread containing sodium iron pyrophosphate.

SUMMARY

Sodium iron pyrophosphate, administered to rats maintained on a diet of whole milk, has been found to have an availability of considerably less than 50% as compared to the utilization of ferrous or ferric sulfate. Likewise, when enriched bread containing sodium iron pyrophosphate or ferrous sulfate was fed to anemic rats, the availability of the former compound was less than 50% of that of the ferrous sulfate.

After this paper was submitted, an article appeared by Nakamura and Mitchell ('43), which calls for comment. These workers reported that the utilization of iron as sodium iron pyrophosphate or ferric chloride is equally satisfactory as tested in anemic rats. It is difficult to reconcile these findings with those reported here. Mitchell ('43) has pointed out that in their experiments the iron compounds were administered daily, in contrast to the thrice-weekly administration of the iron salts in the present study. However, while this might account in part for the difference observed when the iron salts were administered as such, it would not explain the poor utilization of sodium iron pyrophosphate when fed in enriched bread ad libitum. It should also be pointed out that while the preparations of sodium iron pyrophosphate were obtained from the same manufacturer, they were, of course, different samples, and this might to some extent account for variation in degree of utilization. No doubt further work will clarify this matter.

LITERATURE CITED

CEREAL CHEMISTRY LABORATORY METHODS FOURTH EDITION 1941 Am. Ass. Cereal Chemists, Lincoln, Nebraska, p. 48.

ELVEHJEM, C. A. 1942 Personal communication.

ELVEHJEM, C. A., E. B. HART AND W. C. SHERMAN 1933 The availability of iron from different sources for hemoglobin formation. J. Biol. Chem., vol. 103, p. 61–70.

ELVEHJEM, C. A., AND A. R. KEMMERER 1931 An improved technique for the production of nutritional anemia in rats. J. Biol. Chem., vol. 93, p. 189–195.

LUNDSGAARD, C., AND E. MÖLLER 1922 On the determination of the total oxygen combining power of blood in the Van Slyke apparatus. J. Biol. Chem., vol. 52, p. 377–378.

THE MERCK INDEX, FIFTH EDITION 1940 Merck and Company, Rahway, N. J.

MITCHELL, H. H. 1943 Personal communication.

NAKAMURA, F. I., AND H. H. MITCHELL 1943 The utilization for hemoglobin regeneration of the iron in salts used in the enrichment of flour and bread. J. Nutrition, vol. 25, pp. 39–48.

UNDERWOOD, E. J. 1938 A comparison of ferrous and ferric iron in the nutrition of the rat. J. Nutrition, vol. 16, p. 299–308.

UNITED STATES OF AMERICA PHARMACOPOEIA 1936 Eleventh Revision. Mack Printing Company, Easton, Pa.

PREVENTION OF PEROSIS AND DERMATITIS IN TURKEY POULTS [1]

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It is recognized that manganese is necessary for the prevention of perosis in chicks (Wilgus, Norris and Heuser, '37); however, Jukes ('40) found both manganese and choline essential for protection of poults from perosis. Jukes ('40), Hogan et al. ('41) and Record and Bethke ('42) established the essential rôle of choline in the prevention of perosis in chicks.

It was recently reported that in addition to choline, there exists at least two other organic nutrients concerned in the prevention of perosis in chicks. One of these substances is biotin (Jukes and Bird, '42), and the other substance (Richardson and Hogan, '41), not identical with any of the recognized vitamins, is present in the aqueous extract of desiccated beef liver. This unrecognized substance was adsorbed on fuller's earth and subsequently eluted with ammonium hydroxide. Perosis has been reported to accompany the egg white injury syndrome in chicks (McElroy and Jukes, '40), which suggests that this symptom is a result of induced biotin deficiency.

Hegsted et al. ('40, '42) and Ansbacher and Landy ('41) established the essential rôle of biotin in the prevention of a scaly dermatitis in chicks which was distinct from the dermatitis produced by pantothenic acid deficiency. Lepkovsky and Jukes ('36) and Jukes ('38) reported that vitamin G was an

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'anti-dermatitis' vitamin in poult nutrition. Patrick, Boucher, Dutcher and Knandel ('41) reported that poults receiving a simplified ration containing a yeast residue or crystalline biotin methyl ester did not develop dermatitis even when the ration was grossly inadequate in riboflavin. Supplementation with 8 μg. of biotin daily gave complete protection. Perosis was frequently observed in this laboratory in turkey poults which received rations adequate in choline and manganese. Dried brewers' yeast was found to be effective in preventing perosis under these conditions. This paper reports studies of the anti-perosis properties of yeast fractions, crystalline biotin methyl ester and choline. Further evidence on the effectiveness of biotin and ineffectiveness of riboflavin in the prevention of dermatitis is also presented.

EXPERIMENTAL

The composition of the simplified basal ration is given in table 1. Bronze and White Holland turkey poults were used throughout the experiment. They were started when 1 day old and kept in electrically heated brooders equipped with

TABLE 1

Composition of the simplified basal ration.

	%
Purified casein	25
Extracted wheat bran	20
Salt mixture no. 30 [1]	4
Manganese sulfate	0.04
Soybean oil [2]	5
Cod liver oil (400 D)	2
Wheat germ oil [3]	2
Polished rice	5
Choline	0.2
Pyridoxine	0.0005
Thiamine	0.0005
Vitamin carriers as described in tables 2 and 3	
Corn starch to make 100%	

[1] Hubbell, Mendel and Wakeman ('37).
[2] Kindly furnished by Central Soya Co., Fort Wayne, Indiana.
[3] Kindly furnished by General Mills Inc., Minneapolis, Minnesota.

hardware cloth floors. The experimental ration and water were fed ad libitum.

The vitamin carriers were prepared from strain S dried brewers' yeast [2] as follows: (1) The yeast was extracted five times with 95% ethyl alcohol at 65°C. (2) The alcohol insoluble fraction was extracted ten times with boiling HCl-acidulated water at pH 4. (3) The residue was not treated further. (4) The acidulated aqueous extract from 2 was concentrated in vacuo and fed as such or fractionated in the following manner: (A) Alcohol-precipitate and filtrate-fraction: these fractions were prepared by adding enough 95% ethyl alcohol to obtain maximum precipitation and then separated by decantation. (B) Norite and fuller's earth eluates: these fractions were prepared by treating the acidulated aqueous extract, after being concentrated to a convenient volume, with norite or fuller's earth and eluting the adsorbates with 0.2% ammonium hydroxide, thus forming the norite or fuller's earth eluate.

The extracted wheat bran was prepared as follows: (1) Washed once with tap water at 60°C. (2) Washed five times with 0.1% ammonium hydroxide at 60°C. (3) Washed eight times at 60°C. with HCl-acidulated water at pH 4. (4) Washed with cold tap water until free of acid and then dried in a forced draft oven at 80°C.

Biotin concentrate I was prepared from liver residue according to the method of du Vigneaud et al. ('41), and biotin concentrate II (S.M.A.-200) and crystalline biotin methyl ester were purchased from a commercial concern.[3]

The influence of riboflavin on dermatitis and perosis

A summary of results obtained by feeding varying levels of riboflavin is presented in table 2.

Basal ration 1 had the following precentage composition: simplified basal 92 (table1), alcohol precipitate of yeast fraction 3, feeding cane molasses 5, and nicotinic acid 0.001. The

[2] Kindly furnished by Anheuser-Busch, Inc., St. Louis, Missouri.
[3] S.M.A. Corporation.

alcohol precipitate of yeast fraction was added as a source of unrecognized vitamins in which the simplified basal ration was likely to be deficient. The feeding cane molasses was added as a source of pantothenic acid. The complete ration was assayed for riboflavin in two laboratories by the fluorometric method: the laboratory of the Cooperative G. L. F. Exchange, Inc., Buffalo, N. Y. reported 155 μg. per 100 gm.; 160 μg. per 100 gm. was found in this laboratory.

TABLE 2

Effect of graded levels of riboflavin on dermatitis and perosis.

SUPPLEMENT TO BASAL RATION 1 [1]	NO. OF POULTS	RESULTS AT 4 WKS. OF AGE	
		DERMATITIS	PEROSIS
		%	%
Riboflavin			
None	16	87	25
200 μg.	12	92	50
230 μg.	17	76	53
260 μg.	16	75	56
290 μg.	18	72	33
320 μg.	18	67	28
350 μg.	15	67	67
380 μg.	11	45	36
380 μg. plus 10% dried brewers' yeast	15	none	none

[1] Riboflavin expressed as micrograms per 100 gm. of ration.

Severe dermatitis and perosis occurred frequently by the end of the third week in the poults of all groups receiving varying levels of riboflavin, while a control group of poults receiving 10% dried brewers' yeast developed normally. The dermatitis involved the feet, anus, mouth, eyes, and most proximal portion of the upper beak. The accumulation of exudate and the severity of the dermatitis around the eyes and mouth may have been aggravated by a secondary infection. In many instances, when the poults lived long enough, the surface of the skin appeared scaly and felt like parchment and the wings became rigid, due to dermatitis involving the skin of this area, while hemorrhagic fissures appeared in the dermis of the feet. The quantity of riboflavin in the ration did

not appear to influence the time of onset or incidence of either dermatitis or perosis. The type of perosis present was dissimilar to that observed in poults receiving rations low in choline or manganese. The legs appeared weak and although the tendon of Achilles seldom slipped permanently from its condyle, the outward manifestation was a wobbly gait and a loose-joint appearance in the hock joints. The poults stood with their hocks further apart than normal and the angle formed by the tibia and metatarsus was increased until a 'peg-leg' appearance resulted.

The use of choline, biotin, and yeast fractions in the study of dermatitis and perosis

Following the experiments which showed that riboflavin did not influence the incidence of dermatitis, the work was extended to include the rôle of biotin, choline and various fractions of yeast in turkey nutrition. These results as related to dermatitis and perosis are recorded in table 3.

Poults receiving rations adequate in biotin but inadequate in any fraction of the aqueous extract of yeast or in riboflavin were protected from dermatitis but not from perosis. The alcohol-precipitate fraction when fed as the only vitamin concentrate added to the basal ration was ineffective in the prevention of perosis or dermatitis. A combination consisting of a biotin concentrate with either the ammonium hydroxide eluate or the alcohol-precipitate fraction was required to protect the poults from perosis. A ration containing 30 parts of untreated fresh egg white and 70 parts of a practical ration produced perosis and dermatitis in poults. The incidence of perosis did not always parallel the incidence of dermatitis in poults receiving rations deficient in biotin. This agrees with the report of Jukes and Bird ('42) that with chicks a smaller intake of biotin is required to prevent perosis than is required to prevent dermatitis. The above authors reported that 0.34 μg. of biotin per chick per day was enough to completely protect the chick from perosis but not enough for protection against dermatitis. We were unable to show that inositol either increased

or decreased the incidence of perosis; however, choline, when fed at a level of 0.2% in addition to 10% strain G dried brewers' yeast [4], gave complete protection from perosis. A

TABLE 3

Effect of choline, biotin and yeast fractions on dermatitis and perosis.

SUPPLEMENT TO BASAL RATION 2 [1]	NO. OF POULTS	RESULTS AT 4 WKS. OF AGE	
		DERMATITIS	PEROSIS
		%	%
Strain G yeast			
Without choline	18	0	78
Plus 0.05% choline	12	0	33
Plus 0.10% choline	10	0	30
Plus 0.15% choline	9	0	11
Plus 0.20% choline	17	0	0
Basal only	8	100	50
Yeast residue	30	0	53
Without riboflavin	22	0	45
Plus aqueous extract of yeast	26	0	0
Plus norite eluate	18	0	0
Plus norite eluate plus inositol	17	0	0
Plus fuller's earth eluate	10	0	0
Plus inositol	16	0	56
Plus 1% salt mixture no. 30	17	0	47
Aqueous extract of yeast	10	60	0
Biotin II	6	0	50
Alcohol precipitate	31	84	71
Plus biotin I	10	0	0
Plus biotin II	10	0	0
Plus crystalline biotin methyl ester [2]	5	0	0
Aqueous extract of yeast			
Plus biotin I	11	0	0
Plus biotin II	12	0	0
Practical ration	10	0	0
Plus 30% raw egg white	10	70	60

[1] Basal ration 2 was composed of the simplified basal ration (table 1) plus 200 μg. Ca-pantothenate, 500 μg. riboflavin and 200 mg. choline per 100 gm. of ration, unless modified as indicated in the body of the table.

[2] Crystalline biotin methyl ester was fed by pipette at a level of 2.5 μg. on the 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 18, and 21 days of age.

[4] Kindly furnished by Anheuser-Busch, Inc., St. Louis, Missouri.

combination of choline, biotin and a factor or factors which can be adsorbed on norite or fuller's earth and eluted with ammonium hydroxide appear to be required for prevention of perosis in turkey poults.

SUMMARY

1. Riboflavin did not influence the incidence of dermatitis in turkey poults.

2. Biotin was found to be an anti-perosis and an anti-dermatitis factor.

3. The anti-perosis property of choline was confirmed.

4. Inositol did not influence the incidence of perosis.

5. One or more factors present in an eluate from an adsorbate of an aqueous extract of dried brewers' yeast possessed anti-perosis properties.

6. It appears, therefore, that at least three organic factors are required to protect the poult from perosis, namely, choline, biotin and an unrecognized factor or factors which can be adsorbed on fuller's earth or norite and subsequently eluted with ammonium hydroxide.

LITERATURE CITED

ANSBACHER, S., AND M. LANDY 1941 Biotin and scaly dermatosis of the chick. Proc. Soc. Exp. Biol. and Med., vol. 48, p. 3.

DU VIGNEAUD, V., K. HOFMANN, D. B. MELVILLE AND PAUL GYÖRGY 1941 The isolation of biotin (vitamin H) from liver. J. Biol. Chem., vol. 140, p. 643.

HEGSTED, D. M., R. C. MILLS, G. M. BRIGGS, C. A. ELVEHJEM AND E. B. HART 1942 Biotin in chick nutrition. J. Nutrition, vol. 23, p. 175.

HEGSTED, D. M., J. J. OLESON, R. C. MILLS, C. A. ELVEHJEM AND E. B. HART 1940 Studies on a dermatitis in chicks distinct from pantothenic acid deficiency. J. Nutrition, vol. 20, p. 599.

HOGAN, A. G., L. R. RICHARDSON, H. PATRICK AND H. L. KEMPSTER 1941 Perosis due to a vitamin deficiency. J. Nutrition, vol. 21, p. 327.

HUBBELL, R. B., L. B. MENDEL AND A. J. WAKEMAN 1937 A new salt mixture for use in experimental diets. J. Nutrition, vol. 14, p. 273.

JUKES, T. H. 1938 The vitamin G requirements of young poults. Poultry Sci., vol. 17, p. 227.

———— 1940 Effect of choline and other supplements on perosis. J. Nutrition, vol. 20, p. 445.

JUKES, T. H., AND F. H. BIRD 1942 Prevention of perosis by biotin. Proc. Soc. Exp. Biol. and Med., vol. 49, p. 231.

LEPKOVSKY, S., AND T. H. JUKES 1936 The response of rats, chicks and turkey poults to crystalline vitamin G (flavin). J. Nutrition, vol. 12, p. 515.

MCELROY, L. W., AND T. H. JUKES 1940 Formation of the anti egg-white-injury factor (biotin) in the rumen of the cow. Proc. Soc. Exp. Biol. and Med., vol. 45, p. 296.

PATRICK, H., R. V. BOUCHER, R. ADAMS DUTCHER AND H. C. KNANDEL 1941 Biotin and prevention of dermatitis in turkey poults. Proc. Soc. Exp. Biol. and Med., vol. 48, p. 456.

RECORD, P. R., AND R. M. BETHKE 1942 Further observations on choline and yeast in chick nutrition. Poultry Sci., vol. 21, p. 271.

RICHARDSON, L. R., AND A. G. HOGAN 1941 The anti-perosis complex. Proc. Soc. Exp. Biol. and Med., vol. 48, p. 459.

WILGUS, H. S. JR., L. C. NORRIS AND G. F. HEUSER 1937 The role of manganese and certain other trace elements in the prevention of perosis. J. Nutrition, vol. 14, p. 155.

STUDIES ON THE NUTRITIONAL REQUIREMENTS OF THE RHESUS MONKEY [1]

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TWO FIGURES

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A large part of our present knowledge of nutrition has been obtained through the use of small experimental animals. Early studies with rats, mice, chicks, and guinea pigs made it possible to differentiate between the fat soluble and the various water soluble vitamins. Dogs, calves, and pigs have been used in the more recent investigations and work with these animals has aided in estimating the human requirements for the various food factors. Experiments on induced vitamin deficiencies in humans have done much to clarify the clinical picture of frank avitaminosis as well as the subclinical type of nutritional deficiency, but such studies are obviously limited. It would seem probable that additional knowledge of the nutrition of man could be obtained through the use of a more closely related animal, namely, the monkey.

The literature dealing with nutritional experiments in which monkeys have been used is not extensive. Early experiments dealt with the production of a pernicious anemia-like syndrome in monkeys (Wills and Bilimoria, '32) by feeding a diet now known to be deficient in several factors. More recent experiments by this same group of workers (Wills, Clutterbuck and

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Evans, '37; Wills and Stewart, '35) have shown that some factor as yet unidentified is concerned in the production and cure of certain anemias, primarily of the pernicious type. The most complete work within the past several years has been that of Day and his collaborators. In a series of papers (Day et al., '35; Day et al., '38; Langston et al., '38; Day et al., '40) evidence has been presented that monkeys fed a modification of the classical blacktongue producing diet of Goldberger (Goldberger et al., '28) fail to survive and are incapable of maintaining a normal blood picture, the distinguishing feature being the abnormally low white cell count. These investigators postulate that some factor in yeast or in liver, which they call "vitamin M," is required by these animals for maintaining normal growth, mouth structure and blood elements.

Other work on the nutrition of the monkey has been reported by Topping and Fraser ('39) who described the mouth lesions associated with dietary deficiencies in this animal. Tomlinson ('39) has presented the symptomatology and histopathology of the mouth lesions in acute vitamin A, vitamin D, nicotinic acid and riboflavin deficiencies. Fraser ('42) has described the chronic deficiency of calcium, vitamin C and a combination of the two deficiencies in monkeys, and Fraser and Topping ('42) have described mouth lesions associated with these deficiencies. Experimental beriberi and spontaneous polyneuritis have been observed in Macaca sylvanus by Leblond and Chaulin-Serviniere ('42). Although they were not primarily concerned with nutritional studies, Johnstone and Reed ('37) attempted to produce a "vitamin G" deficiency. Harris ('37) differentiated the antiblacktongue and the P–P factors from lactoflavin and vitamin B_6 through the use of monkeys. Several older papers dealing with dietary studies in monkeys have appeared in the literature but many of these have been reviewed in the literature cited. Monkeys have been used for experiments designed to study some phase of physiology, bacteriology, immunology, or pharmacology, but in many cases the workers have neglected to consider the effect of nutrition on their experiments.

One phase of work in this laboratory deals with the role of nutrition in the susceptibility to poliomyelitis and perhaps other infectious diseases, but before setting out on a comprehensive study of the importance of nutritional resistance to infection, it was necessary to determine the normal nutritional requirements of monkeys. No complete study has been reported on the use of the "synthetic diet" in the feeding of monkeys. Previous investigations in this laboratory (Henderson, McIntire, Waisman and Elvehjem, '42) have shown that rats are able to grow and reproduce on such so-called "synthetic diets." Extensive work has also shown that guinea pigs (Sober, Mannering, Cannon, Elvehjem and Hart, '42) and chicks are unable to survive on diets similar to the one to be described below.

EXPERIMENTAL

Each monkey was kept in a roomy individual cage, equipped with both a small sliding door and a larger front door, a screen floor and a removable litter tray. The feed and water were furnished in 1 pound glazed porcelain butter jars suspended in holders under the smaller door, thus keeping the food off the floor and avoiding breakage of the feed cups and excessive waste of the food. All cages and other equipment used in these experiments were kept clean by frequent washing and steaming. The animals were trained to go into a tared weighing box and were thus weighed to within 5 gm. whenever it was deemed desirable to secure body weight data. Usually the monkeys were weighed twice weekly, or more often as indicated by the experimental procedure, specifically during nutritional failure or remission of symptoms because of the therapy being administered.

The diet (M-3) used throughout these studies consisted of sucrose 73 parts, purified vitamin-free casein [2] 18, salt mixture [3] 4, corn oil 3, cod liver oil 2. The vitamin supplements

[2] Both Smaco-vitamin free casein (SMA Corp.) and Labco-vitamin free casein (Borden Company) were used with equal success.

[3] Phillips, P. H., and E. B. Hart. J. Biol. Chem., vol. 109, p. 657, 1935.

were given to each monkey in the water dish at the day's first feeding in a minimum amount of water, slightly sweetened to improve palatability. The daily vitamin supplements contained the following: thiamine hydrochloride 1 mg., nicotinic acid 5 mg., riboflavin 1 mg., pyridoxine hydrochloride 1 mg., calcium pantothenate 3 mg., choline chloride 50 mg., para-aminobenzoic acid 100 mg., i-inositol 100 mg., and vitamin C 25 mg. The individual vitamin solutions were made up at least once a week, and kept in brown bottles in concentrations that were convenient for easy measurement, so that a particular vitamin could be omitted from the daily supplement when desired. The monkeys usually drank the daily vitamin supplements at once. In no case was water furnished to the animals until the daily supplements were consumed. Adequate quantities of the particular diet were given twice daily; each feeding consisted of 100–200 gm., depending on the size of the monkey. When a diet was altered, the inclusion of the particular addition to the diet was made at the expense of the 95 parts of the mixture of dry ingredients. If the monkeys were given a liver fraction as therapy in an attempt to obtain remission of the loss in weight and specific symptoms, the solution of the liver was given with the vitamin supplements. If the animal did not consume it voluntarily, it was administered by stomach tube, no difficulty being encountered in giving as much as 50 cc. by tube daily. Tube feeding was discontinued as soon as the animal indicated a desire to drink the supplement.

The Rhesus monkeys used in these experiments were obtained through animal dealers and were tested immediately upon arrival with old tuberculin (OT) and with a purified protein derivative (PPD), one preparation in each eyelid subcutaneously. Chest plates were taken of individual monkeys in several groups in addition to the tuberculin tests, but in more recent shipments we have been satisfied with the results of the tuberculin tests only. The animals were retested with the tuberculin preparations at approximately 3–4 month intervals so that the hazard from this disease as well as the loss

to the experiments could be reduced to a minimum. Out of a total of one hundred monkeys on experiment three animals were discarded when the routine checkup indicated that they had the disease, and only three others have come to autopsy showing signs of tuberculosis.

Since the experiments were planned to study growth responses of the animals it was necessary to obtain small healthy monkeys. In no case were monkeys placed on experiment if they weighed as much as 3 kg. or if they were over 2–3 years of age as indicated by observation of their dentition. The starting weights of monkeys ranged from 1 kg. to 2.5 kg., and the age from about 1 to 3 years. Blood examinations were made at frequent intervals throughout the experimental periods. Hemoglobin was determined in the Evelyn photoelectric colorimeter and red and white cell counts were made in the usual manner from blood drawn from the marginal vein of the ear. Stool cultures were taken during the course of the experiment in order to check the presence of intercurrent intestinal infections.

The first experiments were planned so that growth responses in monkeys fed the synthetic diet could be compared with those obtained in animals fed the synthetic diet with a variety of liver fractions. Of the more than one hundred monkeys used in our studies thirteen have been allowed to remain on the basal diet plus the vitamin supplements until death. Of these thirteen, one was in very poor condition when received, and one had tuberculosis. The remaining eleven animals gradually lost weight and died within 28 to 119 days. All eleven animals appeared cachectic, and showed a definite gingivitis with unnatural stains on the teeth, an unkempt blackish appearance of the fur, occasional transient edema of the face, and anorexia. At autopsy each animal showed extensive gingivitis with occasional necrosis of the gums, often involving the mucous lining of the cheek. Four monkeys were able to survive long enough to develop extensive, gangrenous, perforating, necrotic areas of the face which began as infectious foci on the inner cheek. Gross examination of the heart, spleen, kid-

neys, pancreas, adrenals, sex glands and muscle showed no abnormal appearance. The lungs were normal in nearly all cases except for an occasional calcified tubercle or signs of hypostasis usually seen in the highly cachectic animals. The gastrointestinal tract showed petechial hemorrhages throughout, with less involvement of the stomach than the intestines. Tissues from all animals were taken for microscopic examination.

TABLE 1

Growth and blood data of monkeys receiving synthetic diet and of those receiving various liver fractions.

SYNTHETIC DIET PLUS ALL 9 VITAMINS PLUS	NUMBER OF MONKEYS AND SEX	AVERAGE GRAM GAIN PER DAY	RANGE OF GAIN PER DAY IN GRAMS	AVERAGE BLOOD PICTURE		
				Red blood cells per c. mm.	White blood cells per c. mm.	Hemoglobin per 100 ml.
				millions	*thousand*	*grams*
(No supplements)	7 ♂		.83–13.9	4.33	9.66	12.3
	4 ♀		1.76– 8.15	4.40	8.17	12.7
3% whole liver substance	1 ♂	8.45		5.17	18.92	14.4
	1 ♀	6.5		4.53	14.32	13.7
1% liver extract	1 ♂	21.5		4.98	16.21	12.7
	1 ♀	7.65		5.94	19.20	12.6
3% liver extract	7 ♂	5.1	3.3 – 7.9	5.61	19.59	12.8
	5 ♀	7.3	4.2 –14.8	5.54	18.44	12.6
1% solubilized liver residue	2 ♀	7.5	7.0 – 7.9	5.51	12.98	12.5
3% solubilized liver residue	2 ♂	5.6	4.0 – 7.2	5.17	18.55	13.4
	2 ♀	3.1	3.0 – 3.2	5.18	17.09	12.4

The blood picture of monkeys kept on the basal diet indicated that during the onset of the deficiency and at death there was a slight reduction in the number of red blood cells, and a definite drop in the number of white blood cells. Moderate degrees of anemia were observed in those animals receiving the basal deficient diet since the hemoglobin values during the plateau in weight and during acute deficiency were somewhat lower than in those getting whole liver or liver extract. The figures for hemoglobin in table 1 are the averages for the

entire period. The white cell count averaged for the monkeys listed in the table reflects the count during the onset of the deficiency whereas in some monkeys the count actually dropped from 5000 to 2000 the day before death. In one monkey, the white count rose very abruptly prior to death and autopsy showed consolidation of the lungs and the presence of adhesions in the thorax. It seems probable that the white cell count rose in response to the infection after having gone down due to the deficiency. Leucopenia was a consistent finding in the deficient animals and had no bearing on the slight anemia.

All the monkeys fed the basal diet exhibited loose, mucous stools during the period of weight loss and the stool specimens showed positive cultures of Shigella paradysenteriae.

Twenty-two monkeys were given diets containing varying amounts of different liver fractions and table 1 summarizes a portion of the data on these animals. All the animals on the liver diets showed good growth, and are continuing in excellent health and showing characteristic sexual changes. Since many factors influence the response of the individual animal to a particular diet, such as duration of experiment, previous nutritional history, genetic variability, age and weight at start of the experiment, no attempt is made to appraise the relative value of the particular liver fraction, and the table lists only the range of the gain per day to show the variability in growth responses. It is obviously an error to insist that an average of the responses of several monkeys is a true indication of the merit of the liver fraction. The curves shown in figure 1 give typical responses of individual monkeys on the basal diet together with those of animals given whole liver, a water soluble portion of liver (liver extract) and an enzymically hydrolyzed fraction remaining from the water extract of the liver (solubilized liver residue). Several of the monkeys have been kept on the diet plus 3% liver extract for more than 575 days with continued gain in weight. Individual growth records also furnish evidence that whole liver is as effective as liver extract in supporting growth at a 3% level. The solubilized liver residue appears to give a slower rate of

growth than other fractions although the monkey appears to be in a good state of nutrition. Some monkeys kept on solubilized liver for long periods show some definite change in the fur and skin.

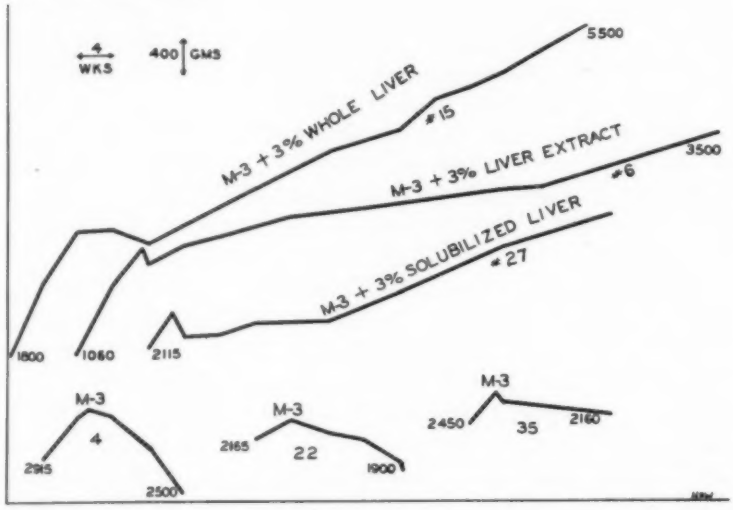

Fig. 1 Typical responses of monkeys on basal synthetic diet and on various liver products.

DISCUSSION

It is evident from these data that monkeys are unable to survive upon a "synthetic diet" containing sucrose, purified casein, mineral salts, corn and cod liver oils, and the known crystalline and readily available members of the vitamin B group, thiamine, riboflavin, pyridoxine, nicotinic acid, choline, calcium pantothenate, inositol, and para-aminobenzoic acid together with vitamin C. The gain in weight in the monkeys fed this diet is only transitory and once the stores in the body are depleted, there is a leveling off of the weight curve followed by a gradual failure with increasing cachexia and final death. The onset of secondary infections, the anemia, leucopenia, and gingivitis are outward indications of upset metabo-

lism and lowering of the natural defenses of the body. The moribund condition is accompanied by anorexia and a cyanotic appearance of the skin, giving the black dull-appearing fur. There is a marked reduction in the normal reflexes of the animal with an accompanying loss of activity. This condition is quickly improved by the feeding of any of the three liver preparations, whole liver, liver extract or solubilized liver residue.

During the course of these experiments it became evident that if a monkey kept on the basal diet was to be saved from certain death, it was necessary to administer one of the liver preparations before too sharp a loss in weight was noted. This procedure was resorted to in more than twenty monkeys, and in several of the animals the curative action of liver was demonstrated two or three consecutive times. In the curative trials, as well as in the prophylactic experiments, 3–5 gm. of any of the three liver fractions administered daily caused remission of symptoms within a period varying from 4 to 21 days. Figure 2 illustrates a typical response of a monkey to liver extract therapy after showing nutritional failure on the basal diet. It should be noted that feeding 20 μg. biotin [4] per day for 60 days during the period of nutritional failure had no effect on the course of the deficiency. The immediate growth response to a short period of liver extract feeding (5 days) is followed by a tendency to drop sharply after a few weeks. Liver therapy again at this point is followed by a sharp gain in weight which persists for a longer period before the plateau occurs, since the liver was given for 14 days. The blood picture is also charted and demonstrates the slight anemia and accompanying lowering of red blood cells during the more severe deficiency state. Since no anemia was present when the weight dropped the second time, the deficiency was apparently not as acute as the previous time. The white cell counts at appropriate intervals are also given in the figure, showing the leucopenia that exists during deficiency and its remission after therapy. The white cells are apparently more sensitive to

[4] Biotin conc. no. 200, SMA Corp., Chagrin Falls, Ohio.

acute deficiency and to therapy as reflected in the respective low and normal counts.

The length of time elapsing between the start of the experiment and the death of monkeys fed the basal diet has varied between rather narrow limits when one considers the number of factors which can influence the results obtained. The average survival time of the eleven monkeys on the basal diet was 88 days; the range was 28 to 119 days. Only three monkeys

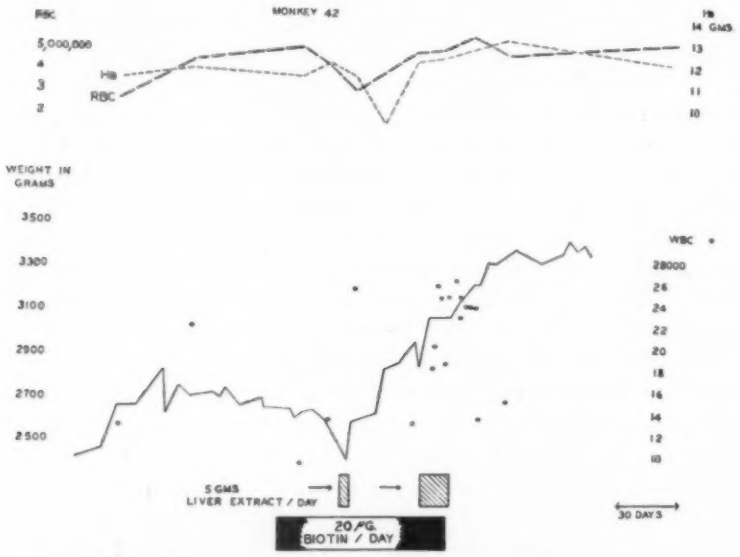

Fig. 2 Typical response of a monkey to liver extract therapy.

survived less than 88 days, the intervals being 28, 53 and 56 days. Although the weight and approximate age of the animal was known at the start of the experiment, the maximum gain in weight differed in the monkeys so that, whereas one animal gained 75 gm. in 91 days before death, another gained 665 gm. in a similar period of 87 days. It should be pointed out that each monkey must be considered individually with a recognition of the genetic factors which may influence the experi-

ments. Throughout the experiments, before each monkey received a particular fraction of liver which was prepared either from liver extract or from solubilized liver residue, the animal received the basal diet until the first drop in weight. In this way each animal served as its own control and one could judge the growth response with more reliability.

The first twenty-five animals which were fed the various diets were given the six well-established crystalline members of the vitamin B group in addition to vitamin C. Later animals received in addition to these i-inositol and para-aminobenzoic acid (PABA). We could observe no difference in the growth of those animals receiving these added vitamins and those which were given the first seven members. Again, no differences were noticeable in those animals fed the various percentages of liver with or without inositol and PABA.

The symptoms observed in our animals during the height of the deficiency may be the result of a single deficiency, but this has as yet not been established. The deficiency may actually be multiple in character and the symptoms observed could be manifestations of a variety of etiological factors.

It is evident that at least one, but more likely several factors required by the monkey are present in the liver. There appear to be no great differences between whole liver and liver extract since the animals grow equally well on both these products. The evidence is accumulating that the solubilized liver residue is not qualtitatively equal to the other liver preparations since a definite change occurs in the animals kept on this diet for a long period. Investigations on this point are being pursued.

From our observations on the animals kept on the basal diet and upon the same animals which have been autopsied, we must conclude that there is an impairment of the natural barriers against infection in these animals. The autopsies have furnished confirmation of this observation since an inflammatory gingivitis of the gum is seen at the teeth margins, and when the infection spreads to adjacent surfaces of the inner cheek ulceration of the tissues results. The various liver preparations are able to prevent or alleviate this condition.

During nutritional failure of several monkeys, the local infection was cured by administration (1 gm./day) of sulfathiazole in the form of the sodium salt. Although the infection cleared, nutritional failure persisted but was delayed somewhat when the invasion processes of the infection were eliminated. An interesting observation was made in the treatment of one deficient monkey given liver extract by stomach tube over a long period. The extensive ulcerated cheek (noma-like condition) was a complicating factor in the nutritional recovery of this monkey. After several 2 gm. daily feedings of sodium sulfathiazole the beneficial effect of the liver extract was visible in that growth was resumed, and even though the sulfa drug was discontinued and the ulcer still purulent, the liver extract apparently aided a return of the natural defenses with the resulting elimination of the infection and the beginning of the repair processes which ultimately completely filled in the cheek.

Further evidence of lowered resistance to infection in nutritional failure was obtained from gross observations of the gastrointestinal tract of monkeys fed the basal diet. Petechial hemorrhages were commonly observed in the mucosa of the entire intestinal tract in deficient animals, together with necrosis and ulceration of the colon in monkeys with frank bacillary dysentery. The presence of dysentery in vitamin deficient animals has been described by other workers (Janota and Dack, '39; Verder and Petran, '37; Topping and Fraser, '39; and Day et al., '40). Cultures of the stools of monkeys during the deficiency state tend to confirm previous reports.

SUMMARY

1. Monkeys are unable to survive upon a purified diet containing sucrose, casein, salts, corn oil, and all eight readily available members of the B group of vitamins and vitamin C.

2. The deficient monkeys show a loss in weight, anorexia, leucopenia, slight anemia, cachexia, and intercurrent infections, especially bacillary dysentery.

3. Any one of three liver products, whole liver, liver extract, and solubilized liver residue when fed at a 3% level in the diet, supports good growth, maintains the normal blood picture and tends to prevent dysentery and other secondary infections.

ACKNOWLEDGMENT

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LITERATURE CITED

DAY, P. L., W. C. LANGSTON AND W. J. DARBY 1938 Failure of nicotinic acid to prevent nutritional cytopenia in the monkey. Proc. Soc. Exp. Biol. and Med., vol. 38, p. 860.

DAY, P. L., W. C. LANGSTON AND C. F. SHUKERS 1935 Leucopenia and anemia in the monkey resulting from vitamin deficiency. J. Nutrition, vol. 9, p. 637.

DAY, P. L., W. C. LANGSTON, W. J. DARBY, J. G. WAHLIN AND V. MIMS 1940 Nutritional cytopenia in monkeys receiving the Goldberger diet. J. Exp. Med., vol. 72, p. 463.

FRASER, H. F. 1942 A chronic deficiency of (1) calcium, (2) vitamin C and (3) both calcium and vitamin C in monkeys. U. S. Pub. Health Reports, vol. 57, p. 959.

FRASER, H. F., AND N. H. TOPPING 1942 Mouth lesions in monkeys associated with a chronic deficiency of (1) calcium, (2) vitamin C and (3) both calcium and vitamin C. U. S. Pub. Health Reports, vol. 57, p. 968.

GOLDBERGER, J., G. A. WHEELER, R. D. LILLIE AND L. M. ROGERS 1928 A study of the black-tongue preventative action of sixteen foodstuffs with special reference to the identity of black-tongue of dogs and pellagra of man. U. S. Pub. Health Reports, vol. 43, p. 1385.

HARRIS, L. J. 1937 The vitamin B complex. Differentiation of the antiblack-tongue and the P P factors from lactoflavin and vitamin B_6. VII. Experiments with monkeys and other species. Biochem. J., vol. 31, p. 1414.

HENDERSON, L. M., J. M. MCINTIRE, H. A. WAISMAN AND C. A. ELVEHJEM 1942 Pantothenic acid in the nutrition of the rat. J. Nutrition, vol. 23, p. 47.

JANOTA, M., AND G. M. DACK 1939 Bacillary dysentery developing in monkeys on a "vitamin M" deficient diet. J. Inf. Diseases, vol. 65, p. 219.

JOHNSTONE, H. G., AND A. C. REED 1937 Studies on vitamin G deficiencies in monkeys. Am. J. Trop. Med., vol. 17, p. 619.

LANGSTON, W. C., W. J. DARBY, C. F. SHUKERS AND P. L. DAY 1938 Nutritional cytopenia (vitamin M) deficiency in the monkey. J. Exp. Med., vol. 68, p. 923.

LEBLOND, C. P., AND J. CHAULIN-SERVINIERE 1942 Spontaneous beriberi of the monkey as compared with experimental avitaminosis. Am. J. Med. Sciences, vol. 203, p. 100.

SOBER, H. A., G. J. MANNERING, M. D. CANNON, C. A. ELVEHJEM AND E. B. HART 1942 Nutrition of the guinea pig. J. Nutrition, vol. 24, p. 503.

TOMLINSON, T. A. 1939 Oral pathology in monkeys in various experimental dietary deficiencies. U. S. Pub. Health Reports, vol. 54, p. 431.

TOPPING, N. H., AND H. F. FRASER 1939 Mouth lesions associated with dietary deficiencies in monkeys. U. S. Pub. Health Reports, vol. 54, p. 416.

VERDER, E., AND E. PETRAN 1937 Vitamin A deficiency in the rhesus monkey — studies on the gastro-intestinal tract, the blood and nervous symptoms. J. Inf. Diseases, vol. 60, p. 193.

WILLS, L., AND H. S. BILIMORIA 1932 Studies in pernicious anemia of pregnancy. V. Production of a macrocytic anemia in monkeys by deficient feeding. Indian J. Med. Research, vol. 20, p. 391.

WILLS, L., AND A. STEWART 1935 Experimental anemias in monkeys, with special reference to macrocytic nutritional anemia. British J. Exp. Path., vol. 16, p. 444.

WILLS, L., P. W. CLUTTERBUCK AND B. D. F. EVANS 1937 A new factor in the production and cure of macrocytic anemias and its relation to other hemopoietic principles curative in pernicious anemia. Biochem. J., vol. 31, p. 2136.